DOSAGE COMPENSATION IN *DROSOPHILA MELANOGASTER* TRIPLOIDS. II. GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY

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ABSTRACT

The level of activity of the enzyme glucose-6-phosphate dehydrogenase was determinel in flies having seven different chromosomic constitutions. All those having an integral number of chromosomes [*XAA*, *XXAA*, *XAAA*, *XXAAA*, and *XXXAAA* (*X*=*X* chromosome, *A*=set of autosomes)] were found to have similar units of enzyme activity/mg live weight, while diploid females with a duplication and triploid females with a deficiency showed dosage effect. The amount of enzyme activity *per cell*, on the other hand, is also independent of the number of *X*'s present but appears roughly proportional to the number of sets of autosomes.——It is proposed that dosage-compensated sex-linked genes are controlled by a positively acting regulatory factor(s) of autosomal origin. With this hypothesis it is possible to explain dosage compensation as a consequence of general regulatory mechanisms without invoking a special device which applies only to the *X* chromosomes.

THE classical observations that the expression of many sex-linked mutants is quantitatively similar in males and females of *Drosophila melanogaster* despite inequality in the number of *X* chromosomes have been extensively supplemented and refined in recent years. Earlier work on dosage compensation was reviewed by MULLER (1950) and STERN (1960). Examples of more recent studies of this problem are the papers of KORGE (1970), CHATTERJEE and MUKHERJEE (1971), and TOBLER, BOWMAN and SIMMONS (1971).

In *D. melanogaster*, chromosomal constitutions other than one *X* and two sets of autosomes (1*X*2*A*) or two *X*'s and two sets of autosomes (2*X*2*A*) occur but these have been studied only fragmentarily. For instance SEECOF, KAPLAN and FUTCH (1969) reported that two *X*-coded enzymes (6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase) appear to be equally represented in triploid (3*X*3*A*) and diploid (2*X*2*A*) females when their activity is expressed as enzyme units per mg of live weight.

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[2] The term "class of flies" is used throughout this paper to mean specifically flies having identical genotypes and genetic history. Flies having the same number of *X* chromosomes and autosomal sets, regardless of their genotype are said to be of the same "chromosomic constitution".

[3] Exceptions were two metamales found on separate occasions. These, as well as sib sisters used for comparison, were assayed individually at a concentration lower than 2.5 mg/ml.

The present study is an attempt to obtain a more complete picture of the expression of the *X* chromosome in flies of various chromosomal constitutions. In our previous work, we found that the autoradiographically measured rate of RNA synthesis in polytene *X* chromosomes of triploid intersexes (*2X3A*) approaches that observed in triploid females (*3X3A*) (MARONI and PLAUT 1972 and 1973). This observation of dosage compensation in comparing *2X3A* and *3X3A* individuals and SEECOF, KAPLAN and FUTCH's data for *2X2A* and *3X3A* flies led us to suggest that dosage compensation, as well as the different dosage phenomena observed with deletions and duplications (STERN 1960), are the result of the action of regulatory elements of autosomal origin that are necessary for activation of the transcriptive process; many sex-linked genes would share a common species of regulatory factor, and with respect to the amount of factor present there would be an excess of nonactivated gene sites. It is a consequence of this hypothesis that (a) if the number of autosomal sets is kept constant, the total amount of a certain gene's product per cell will be the same regardless of the number of whole *X* chromosomes present, (b) this amount of gene product will be proportional to the number of autosomal complements, and (c) if the number of autosomal complements and the number of *X* chromosomes are kept constant, the amount of gene product per cell will be proportional to the gene dose (variations in gene dosage accomplished through deletions or duplication).

In this second part of our study, we have determined the activity of glucose-6-phosphate dehydrogenase (G-6-PD) in crude extracts of triploid and diploid flies. Assuming that the measured activity is proportional to the actual amount of enzyme present, it may be interpreted as a reflection of the extent of gene expression. The structural gene for G-6-PD maps in the *X* chromosome in position 63.0; its two alleles, Zw^A and Zw^B, produce enzymes of different electrophoretic mobility and specific activity (YOUNG, PORTER and CHILDS 1964; STEELE, YOUNG and CHILDS 1969).

We observed that triploid flies have comparable amounts of enzyme, regardless of whether they have 1, 2, or 3 *X*'s and, furthermore, that the amount of enzyme per cell in triploids is approximately 1.5 times that found in diploids. Thus all observations, including those on deletion and duplication, fall within the expectations of our working hypothesis.

MATERIALS AND METHODS

D. melanogaster stocks

Stock *a*: *C(1) RM,y²sc w^aec/FM6*, triploid females and *FM6/B^S·Y·y+* diploid males. This triploid stock produces, in addition to the parental types, triploid intersexes, *FM6/FM6* females, and *C(1)RM,y²sc w^aec* attached-*X* females.

Stock *b*: *C(1)RM,y²sc w^aec/B^S·Y·y+* females and *y²sc w^aec/B^S·Y·y+* males. The *X* chromosome in males was derived from the main portion of one of the attached *X*'s by crossing over in a triploid female obtained from stock *a*.

Stock c: *T(1,4)B^S (16A1), y² cv v B^S car* males and *C(1)DX,y f* females. The small proximal fragment of the male *X* chromosome (hereafter designated *Dp(X)*) carries *Zw* and segregates independently from the main portion of the *X* which is translocated to the fourth chromosome. (We will refer to this deficient *X* as *Df(X)*). In crosses with appropriate females it is possible to obtain flies carrying a deletion or a duplication for *Zw*.

Stock *d*: $w^a spl$; *C(2L), dp*; *C(2R),px*; *C(3L)*, +; *C(3R)*, +. Homologous autosomal arms are attached to a common kinetochore (compound autosomes).

Stock *e*: *C(1)y pn* females and *FM6* males. A cross of *d* males to *e* females will produce triploid individuals as the only viable progeny (For details see MARONI and PLAUT 1973).

Stocks *a* and *c* were obtained from the collection of Oak Ridge National Laboratory, stock *e* from the California Institute of Technology and stock *d* was generously provided by DR. E. GRELL. A complete description of the genetic symbols can be found in LINDSLEY and GRELL (1968).

Table 1 outlines the origin of the various classes of flies[2] used for our analyses; these are numbered for further reference in this paper. All cultures were grown in LEWIS's (1960) standard Drosophila medium at 25°C. Zero- to 12-hr-old flies were collected and aged for 12–14 hours in foodless vials provided with moist paper. At the end of this period they were etherized lightly and immediately weighed, homogenized and assayed for enzyme activity.

Polyacrylamide gel electrophoresis: In order to eliminate systematic errors in the comparative estimates of enzyme activity resulting from the presence of different *Zw* alleles; several potentially interesting stocks were surveyed for the electrophoretic variant present.

Disc electrophoresis in polyacrylamide gels was done according to DAVIS (1964) and the gels were developed by precipitation of formazan dyes following, in general, the method used by YOUNG, PORTER and CHILDS (1964).

Two forms of G-6-PD with the patterns described by STEELE, YOUNG and CHILDS (1969) for the forms A and B were observed. Flies that had only the allele Zw^B were used for measurements of enzyme activity. The Zw^A allele was found in Oregon-R wild strain and the *FM6* balancer chromosomes present in triploid females and males of stock *a*.

TABLE 1

Origin of the Different Classes of Flies Studied

Cross*	Analyzed progeny: X chromosomes	Constitution† X	Constitution† A	Sex	Class	Experiment
3N F(a) x	C(1)RM,y^2sc w^a ec	X	AAA	F	1	1
M(a)	C(1)RM,y^2sc w^a ec	X	AA	I	2	
F(b) x	C(1)RM,y^2sc w^a ec	X	AA	F	3	2
M(b)	y^2 sc w^a ec	—	AA	M	4	
	C(1)RM,y^2sc w^a ec/Dp(X)	X+	AA	F	5	
3N F(a) x	C(1)RM,y^2sc w^a ec	X	AA	F	6	3
M(c)	C(1)RM,y^2sc w^a ec/Df(X)/Dp(X)	X+	AAA	F	7	— 4
	C(1)RM,y^2sc w^a ec/Df(X)	X	AAA	F	8	
F(d) x	w^a spl/w^a spl	XX	A	F	9	5
M(d)	w^a spl	—	A	M	10	
F(e) x	C(1) y pn	X	AAB	I	11	6 —
M(d)	C(1) y pn/w^aspl	X+	AAB	F	12	
	w^a spl	—	AB	mM	13	— 7

F, female; M, male; I, intersex; mM, metamale; 3*N*, triploid.

* Letters in parentheses refer to the stocks used.

† *X* chromosomes are shown on the left and each set of autosomes (*A*) is represented by a single bar on the right. Angled bars indicate compound chromosomes, and dots on the bars the *Zw* genes. The classes of flies as well as the individual experiments in which they were directly compared are numbered for future reference.

G-6-PD assay: The homogenization medium was pH 7.5 0.1 M Tris buffer, to which was added NADP for a final concentration of 0.06 mM. All manipulations were done at 0–4°C. Batches of 4–10 flies were homogenized (using a glass homogenizer fitted with a Teflon pestle) in sufficient medium to yield a ratio of 10 mg live weight/ml homogenization medium.[3] Homogenates were allowed to stand for 30 min. before centrifugation at 10,000 g for 15 min. Immediately after centrifugation, the clear supernatants were pipetted out and assays were completed within 30 min. Reextraction of the pellet with fresh buffer either by further homogenization or sonication released only negligible amounts of enzyme activity.

Reactions were carried out in spectrophotometric cuvettes at room temperature (ca 25°C). To initiate the reaction, 0.2 ml of extract was added to 2.3 ml of reaction mixture at pH 7.5. Final concentrations were: Tris buffer 0.1 M, $MgCl_2$ 3 mM, NADP 0.12 mM, glucose-6-phosphate, monosodium salt, (G-6-P) 15 mM. Enzyme activity was monitored as the increase of optical density at 340 nm resulting from reduction of NADP. When G-6-P was omitted, change in OD was reduced to a small fraction of that detected with the complete reaction mixture. Figure 1a shows the entire course of a reaction. The slope, change in OD per unit time, was determined in the 30 sec.–2 min. interval during which the tracing is a straight line. That this slope is proportional to the amount of enzyme added is demonstrated in Figure 1b. A unit of enzyme activity is that which reduces one μmole NADP in one minute, and measurements are expressed in enzyme units/mg fly.

Sampling and statistical analysis: Since the flies used varied considerably in genetic background and since these studies extended over a period of several months, our experiments were designed to provide accurate *relative* values of enzyme activity. Direct comparisons of enzyme units/mg fly were made only between classes of flies having identical genetic history and emerging from the same culture. For any particular comparison of the enzyme activity of two classes, flies from

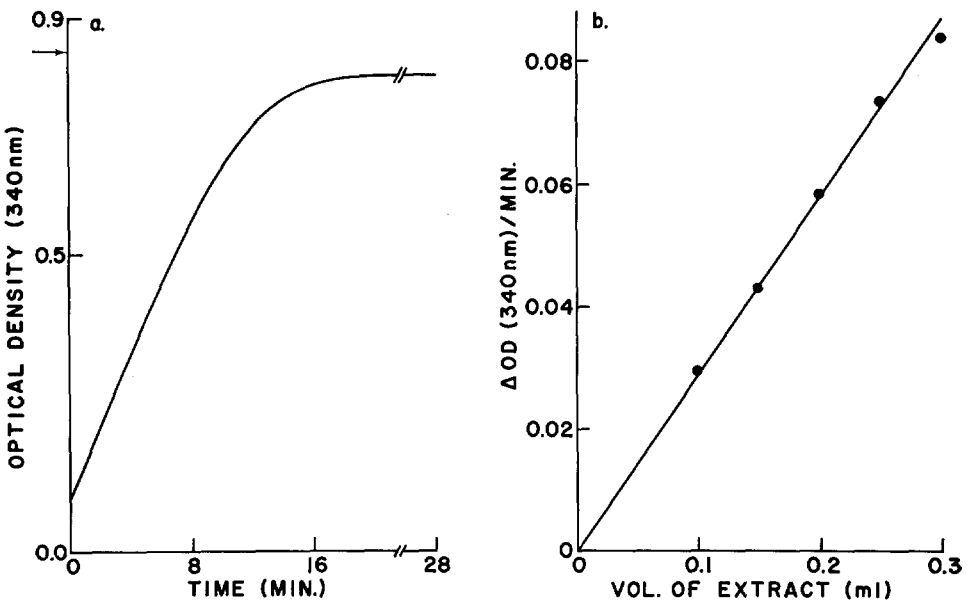

FIGURE 1.—(a) Recorder tracing of an enzymatic reaction allowed to run to completion. The arrow indicates the approximate extinction expected if all the NADP added to the reaction mixture were reduced. The reaction was initiated with 0.2 ml. of extract (24.85 mg/ml) from class 1 females. (b) Enzymatic activity (ΔOD/min) vs quantity of extract assayed. All determinations were done in a total volume of 2.5 ml. Extract used was the same as for Figure 1a.

each were analyzed simultaneously. Several replicas of such paired observations constitute an experiment (Table 1). A ratio was derived from each pair of observations and an average of these ratios, its standard deviation and 95% confidence limits were calculated. The mean value and its standard deviation for each class are also given for each experiment. Differences were tested by the method of the t distribution applied to paired observations. The level of significance used was 5%.

RESULTS

G-6-PD units per mg of live weight: Significant differences in enzyme activity per unit weight were found between most of the classes of flies studied; however, these differences did not exceed 15% when classes having an integral number of X chromosomes were compared. Classes characterized by a deficiency or a duplication involving the *Zw* gene, on the other hand, showed a clear dosage response.

As had been observed before (KOMMA 1966; SEECOF, KAPLAN and FUTCH 1969), males and females are indistinguishable with respect to activity of G-6-PD per mg live weight, diploid and triploid females are very similar and diploid females with a duplication (3 doses of *Zw*) are approximately 40% higher than normal females (Table 2a). We found that triploid females with a deficiency (2 doses of *Zw*) have 54% of the activity observed in normal triploid females (Table 2b).

As compared to those of triploid ($3X3A$) and diploid ($2X2A$) females, the values for intersexes ($2X3A$) are slightly more than 10% higher. Values for the two metamales ($1X3A$) average 14% lower than those of triploid females (Table 2c).

Classes of flies that were analyzed in two different experiments were compared indirectly to each other by normalizing their values to those of classes having a chromosomic constitution that was present in both experiments. That is, the value obtained for Class 11 intersexes was normalized to the value for Class 2 intersexes in order to express the activity of Classes 8, 12 and 13 relative to the activity of diploid females. The last column in Table 2 was constructed in this fashion, giving a value of one to the diploid females.

Amount of G-6-PD per cell: As a measure of gene expression, it would be more meaningful to have an estimate of the amount of enzyme per cell rather than the experimentally determined amount per live weight. In order to express the results on this basis, however, one must take into account the fact that triploid cells are larger than their diploid counterparts (DOBZHANSKY 1929). The finding that the amount of DNA per mg live weight is the same in diploid and triploid females (LUCCHESI and RAWLS 1973a) makes it possible to calculate the magnitude of this size difference. Since nuclei in triploids contain 50% more DNA, cells must be proportionately larger so that the amount of protoplasm per genome remains constant; i.e. per unit weight triploids have 2/3 as many cells as diploids. Therefore, multiplying the activity in triploids by a factor of 1.5 would make the proportionality between enzyme/live weight and enzyme/cell equal in diploids and triploids.

If such a correction is applied, making the relative measures a reflection of

TABLE 2

Glucose-6-phosphate dehydrogenase activity

Experiment*	Class*	Sex and Zw doses†	Chromosomic constitution‡ X	A	Enzyme activity§ U	S_U	R	S_R	Number¶	Differences between classes	Activity‖ relative to $2X2A$
					a						
2	3	F 2	2	2	4.95	0.19	1		13	not	1
	4	M 1	1	2	5.22	0.14	1.07	0.04	13	significant	1.07
5	9	F 2	2	2	4.54	0.52	1		4	not	1
	10	M 1	1	2	4.64	0.47	1.03	0.03	4	significant	1.03
3	5	F 3	2	2	6.75	0.22	1.41	0.03	6	significant	1.41
	6	F 2	2	2	4.80	0.23	1		6	significant	1
	7	F 3	3	3	4.48	0.22	0.93	0.02	6		0.93
					b						
4	7	F 3	3	3	4.76	0.40	1		3	significant	1.01
	8	F 2	3	3	2.53	0.19	0.54	0.06	3		0.54
					c						
1	1	F 2	2	2	3.80	0.48	1		3	significant	1
	2	I 2	2	3	4.26	0.43	1.13	0.03	3		1.13
6	12	F 3	3	3	3.58	0.10	1		8	significant	1.01
	11	I 2	2	3	3.97	0.13	1.12	0.04	8		1.13
7	12	F 3	3	3	3.60	0.08	1		2	not	1.01
	13	mM 1	1	3	3.10	0.16	0.86	0.04	2	applied	0.87

* Defined in Table 1.
† F, female; M, male; I, intersex; mM, metamale.
‡ Number of X chromosomes on the left, and of sets of autosomes on the right.
§ U, mean units of G-6-PD $\times 10^3$ per mg live weight. S_U, standard deviation of the mean. R, mean of the ratios obtained from paired observations. S_R, standard deviation of R.
¶ Number, number of assay replicas in each experiment.
‖ Diploid females were taken as overall standard with an arbitrary value of one.
Note: All values were obtained from 12–26-hour-old individuals. For comparison Class 2 intersexes were also assayed at ages 0–2 days and 2–4 days; values obtained were 3.7×10^{-3} and 3.9×10^{-3} G-6-PD units per mg live weight, respectively. These values fall within the 95% confidence limit of the mean U for 12–26-hour-old Class 2 intersexes.

enzyme activity per cell, the total amount of activity for Zw genes in triploid intersexes is 69% higher than in diploid females despite the fact that both karyotypes have two X's. Also metamales ($1X3A$) are 31% higher and triploid females ($3X3A$) 40% and 52% higher, than diploid females (Table 3).

Figure 2a is a graphical representation of the total activity per cell and Figure 2b a plot of the activity per gene dose as a function of the reciprocal of the number of X's. As can be seen, at each ploidy level total enzyme activity per cell is independent of the number of X's and the values obtained for triploids are approximately 50% higher than for diploids.

TABLE 3

Expected levels of gene expression (transcription) and observed values of G-6-PD activity

Chromosomic constitution	Sex‡	Activity per cell: Expected* — Activity per gene dose × dose	Activity per cell: Expected* — Relative to *XXAA*	Activity per cell: Observed†
XXXAA	mF	$\frac{2}{3}p_i \times 3$	1	
XXAA	F	$p_i \times 2$	1	1
XAA	M	$2p_i \times 1$	1	1.07
XXXAAA	F	$p_i \times 3$	1.5	1.40 1.52
XXAAA	I	$\frac{3}{2}p_i \times 2$	1.5	1.69
XAAA	mM	$3p_i \times 1$	1.5	1.31
Dp(X)XXAA	F	$p_i \times 3$	1.5	1.41
Dp(X)XAA	M	$2p_i \times 2$	2	
Df(X)XAA	F	$p_i \times 1$	0.5	
Df(X)XXAAA	F	$p_i \times 2$	1	0.82

* The expected values were calculated as described in the DISCUSSION.
† The observed values were derived from the last column in Table 2 by multiplying those corresponding to triploids by a factor of 1.5 in order to obtain relative values *per cell.*
‡ mF, metafemale; F, female; M, male; I, intersex; mM metamale.

DISCUSSION

Enzyme activity and gene function: The use of enzyme activity levels as indices of gene function in organisms with different chromosomic constitutions presupposes that the levels observed are the direct consequence of the genetic endow-

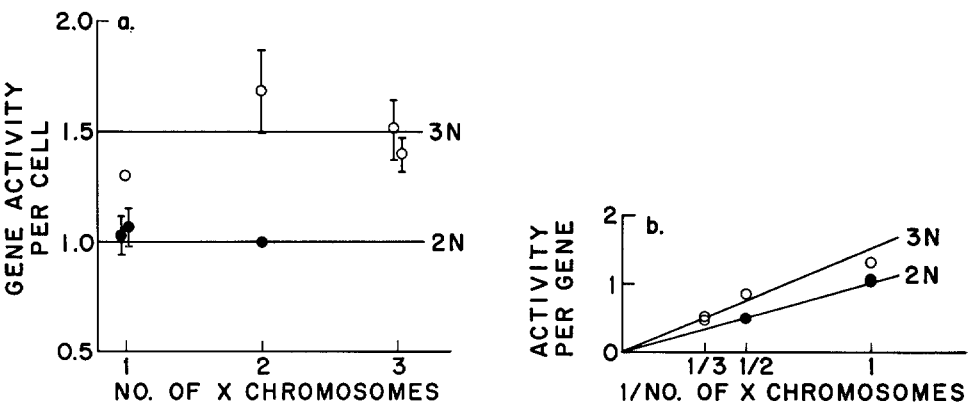

FIGURE 2.—Relative enzyme activities per cell with 95% confidence limits (a) and per gene dose (b). Diploid females were taken as standard with an activity of one for the two gene doses. Solid lines, expected values derived from our hypothesis. ●, diploids (2*N*); ○, triploids (3*N*).

ment of the individuals and do not come about as secondary effects due to physiological and developmental differences imposed by the variations in chromosome complements. This supposition is especially critical in comparisons between aneuploid intersexes and metamales and normal and triploid females. The following considerations are pertinent to an examination of the validity of the data in the proposed context:

1. The specific activity of G-6-PD varies with developmental stage and age (STEELE, YOUNG and CHILDS 1969); intersexes, being slower in preimaginal development might, therefore, be expected to attain maximum levels of enzyme activity later, in the adult stage, than do females. We have found no evidence for such a delay. For instance, analysis of 0–2-day-old and 2–4-day-old intersexes demonstrated that enzyme activity levels found in these flies (Table 2, Footnote) fall within the experimental error of the values given in Table 2 for 12–24-hour-old intersexes. Moreover, enzyme activity per mg live weight is quite similar in intersexes and in diploid and triploid females (Table 2).

LUCCHESI and RAWLS (1973b) observed that the activity of isocitrate dehydrogenase (an autosomally linked enzyme) is quite similar in intersexes and triploid females. This lends further support to the assumption that enzyme levels are not seriously affected by major alterations in the developmental or physiological processes of intersexes.

2. It is known that the intrafly distribution of G-6-PD is not uniform, and that, therefore, differences in tissue constitution between different sexes could affect the interpretation of results. STEELE, YOUNG and CHILDS (1969) observed that the specific activity of G-6-PD is higher in male abdomens than in female abdomens while it is similar in heads and thoraces in the two sexes. The authors ascribed these differences to the presence of eggs (poor in G-6-PD) in the females. In agreement with STEELE, YOUNG and CHILDS's findings we observed that when mature flies (2–4 days old) were used, males could be more than 20% higher than sister diploid females in G-6-PD activity. In an attempt to reduce the confounding effect of eggs in the present study, we used only young individuals (12–24-hour-old) in which mature oocytes are scarce. At this age diploid males and females display indistinguishable values of enzyme activity (Table 2a, Experiments 2 and 5). In the absence of homogeneous cell populations, the ideal material for making these comparisons, we believe that the precautions taken offer some assurance that the data can be meaningfully interpreted.

Finally it should be borne in mind that we are not measuring the rate of synthesis of a direct gene product but, at best, the amount of a protein that is accumulated by young flies. Regulation of this quantity could be accomplished at the transcriptional, translational or post-translational levels. The fact that individuals with genetic deficiencies or duplications show dosage effect, however, seems to indicate that enzyme activity is actually a measure of gene activity in the present instance.

Although it is clear that the influence of physiological and developmental heterogeneity cannot at present be fully evaluated, we feel that the above considerations provide reasonable justification for the interpretation of enzyme

levels in terms of gene activity in intersexes, in diploid and triploid females and in males, where gene dosage phenomena may be manifested. The fact that metamales are rare should be kept in mind, and the limited data available on this form should be interpreted with caution.

Theories of dosage compensation: In a previous communication (MARONI and PLAUT 1973) we discussed the weaknesses of GOLDSCHMIDT's sexuality argument (see also LAKHOTIA and MUKHERJEE 1969) as an explanation for dosage compensation and pointed out that MULLER's hypothesis of sex-linked negative compensators as originally proposed (MULLER 1950), i.e., assuming that the effectiveness of the compensators increases proportionately to the square of their dose, is inconsistent with the observed similarity between triploid females and intersexes.

We would like to present a working hypothesis which we believe can satisfactorily explain the available experimental evidence on dosage compensation. Let us assume that:

1. The rate-limiting factor in the synthesis of X-linked gene products is an element of autosomal origin which is necessary for the transcriptive process.

2. Many sex-linked genes share a common species of this regulatory factor so that all sites belonging to one set or battery of genes compete for a given species of factor.

3. The binding constant of the regulatory molecules is high, and their rate of production is much lower than the maximum potential rate of utilization by the X. Therefore the pool of unbound factors is negligible and there is an excess of free gene sites at all times.

Certain quantitative predictions can be derived from this hypothesis: if n is the number of genes in a set $(G_1 \ldots G_n)$ in an X chromosome, all of which are activated by a certain species of regulatory factor R_G, and r is the amount of R_G produced by a haploid set of autosomes, the rate of synthesis of gene product P_i by one of these genes (G_i) is directly proportional to the total amount of R_G and inversely proportional to n and to the number of times this set of genes is represented in the genome. Thus in a diploid female, for each dose of G_i, the quantity of product synthesized per unit time is:

$$p_i = k\frac{2r}{2n} = k\frac{r}{n}$$

and the total activity per cell of the two homologous genes is

$$2p_i = 2k\frac{r}{n}.$$

For comparison let us choose as a second example a female heterozygous for a deficiency in the G_i locus. If n is relatively large, the activity of the single G_i present in each cell is:

$$k\frac{2r}{2n-1} \simeq k\frac{2r}{2n} = p_i$$

i.e., approximately half of that expected in a normal female. In Table 3, calculations of gene activities for various chromosome constitutions have been made as

outlined above. As can be seen, the values obtained experimentally follow the predicted pattern.

The theoretical values derived for diploid males and females, with and without deletions and duplications, correspond to well-documented phenomena of dosage compensation (STERN 1960; SEECOF, KAPLAN and FUTCH 1969; BAILLIE and CHOVNICK 1971; TOBLER, BOWMAN and SIMMONS 1971). With respect to the triploid flies, the expected and observed values show good agreement. Other experimental findings which fit the hypothesis include our previous autoradiographic determination that the rate of RNA synthesis by *X* chromosomes tends to be equalized in triploid females and intersexes (MARONI and PLAUT 1972 and 1973). Also, LUCCHESI and RAWLS (1973a and b) in a study of triploid and diploid *Drosophila melanogaster*, obtained results quite in agreement with ours: (1) Using a different *X*-linked enzyme, 6-phosphogluconate dehydrogenase (6-PGD), and analyzing heads and thoraces of 24–48-hour-old flies, they observed dosage compensation between $2X3A$ and $3X3A$. (2) In a similar analysis they observed that the activity levels of G-6-PD and 6-PGD *per gene dose* are the same in triploid and diploid females. Since $3X3A$ cells have an extra dose of the genes in question compared to $2X2A$, they concluded that in triploid somatic cells there is a proportional increase (i.e., 50%) in the amount of these enzymes.

Although to our knowledge, no quantitative experimental data are available on metafemales ($3X2A$), we have included them in our table for the sake of completeness. We would expect them to show the same activity as diploid females and males.

Small but reproducible differences are found between most classes for which our hypothesis would predict equal values. These cannot be accounted for by random variations and represent, we believe, the limitations of this method of measurement of gene activity. Conditions which might contribute to such observations have been discussed in the previous section (*Enzyme activity and gene function*). Differences in tissue composition and/or developmental rates are to be expected in flies carrying appreciable duplications or deletions or having chromosomal constitutions widely different from the normal diploid type. Since our hypothesis must necessarily address itself to homogeneous cell populations, all departures from compositional similarity between the classes of flies studied will lead to deviations from the expected values.

Two broad modes of operation of the regulatory mechanism which we propose are possible:

1. If N is the total number of dosage-compensated genes in an X chromosome, it is possible that $n = N$. That is to say, *all* genes in the X would respond to a single species of R_G; R_G would then operate as a potentiator, and a second mechanism responsible for the modulation of specific gene activities would be required.

2. Alternatively, the N genes in the X may be organized in batteries ($n<N$), each battery being comprised of genes which are necessarily activated coordinately and which therefore respond to a battery-specific regulatory factor. In this situation our hypothesis falls within BRITTEN and DAVIDSON's (1969) model of gene regulation in eukaryotes. One aspect which makes the second alternative

particularly attractive is the fact that according to it, dosage compensation is not a special mechanism but rather a manifestation of the general system of gene regulation operating on genes which are functionally grouped. Thus, in order to assure equalization of activity in males and females, the task of the evolutionary process would have been to avoid the existence in both autosomes and sex chromosomes of genes that respond to the same regulatory signals.

Although the agreement between observed and expected values is reasonably good, the proposed mechanism is not the only one which would lead to these expected values. A variation of MULLER's hypothesis is the possibility that the product of sex-linked compensators inhibit the expression of compensated genes according to the mass action law so that gene activity is inversely proportional to the *concentration* of compensator product. Thus in triploid females, for instance, the extra dose of compensators is accompanied by a proportional increase in nuclear volume so that the concentration of compensator product remains the same as in diploid females. Consequently, the rate of transcription *per dose* of a given gene would be unchanged; on a *per cell* basis the total gene activity would be 50% higher.

When this argument is extended to all possible chromosomic constitutions the expected values derived are found to be identical to those obtained by applying the hypothesis we present (Table 3). The inverse linear relationship between activity per gene dose and number of *X*'s and the direct proportionality between gene activity and ploidy (Figure 2) is equally predicted by either conception. MULLER's hypothesis thus modified can be envisioned, as is our model, as the manifestation of a general regulatory mechanism and it too avoids the need for invoking a special device to bring about dosage compensation.

Which of the two hypotheses comes closest to describing the actual mechanism of dosage compensation can only be resolved with further experimentation. In the absence of critical data we feel that ours has a theoretical advantage in that it is based on positively rather than negatively acting controlling elements (See BRITTEN and DAVIDSON [1969] for a discussion of positive controls in eukaryotic cells) and is more amenable to experimental testing through a search for autosomal mutants affecting the expression of sex-linked genes.

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