ON THE MOLECULAR AND CHROMOSOMAL BASIS OF DOSAGE COMPENSATION IN *DROSOPHILA*[1]

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Many sexlinked mutants in *Drosophila* are known to express themselves identically in the two sexes, in spite of the existence of a difference in them in the dosage of genes. This phenomenon, termed by Muller (1932) as dosage compensation, is thought to be accomplished by cancellation of the excess effect in the female by means of complementary plus and minus modifier genes, called compensators. Muller (1950) has shown that w^a/w^a female and w^a/Y male have identical white-apricot eye colour, and w^a/w^a; *tra*/*tra* transformed individuals (the mutant gene *tra* in homozygous condition transforms XX females into phenotypic males, Sturtevant, 1945), have eye colour like that of a w^a/w^a female or w^a/Y male, not as dark as in w^a/dup.w^a male. Thus the dosage compensator genes act as the decisive factors for the identical expression of these sexlinked genes. Furthermore, the 1 dose/male to 2 dose/female relationship is maintained *in toto* in the same way as the 2 dose/male to 4 dose/female for w^a strains specially made by Green (1959, see Stern, 1960). The problem has, however, been complicated through other issues. It is known that even another allele of the same locus, white-eosin (w^e) for example, does not bear similar relationship and the bobbed mutant gene, studied by Stern (1929), fails to show any compensatory effect in the female. Furthermore, it is now thought that compensators may not be restricted to the X-chromosomes but may be present also in the autosomes (Cock, 1964).

Studies on genetic mosaicism in female mammals heterozygous for two sexlinked mutant genes (present in the two homologous chromosomes) and recent works on sex chromatin body in mammals have made the problem of dosage compensation more interesting. In all mammals, one of the two X-chromosomes in female appears heterochromatized in their interphase nuclei. This is thought to be due to a random inactivation of one of the two X's in the female (Lyon, 1962, 1963). Later works have further revealed that sex chromatin is actually "inactivated" from the point of view of final genetic expression (Lyon, 1962) as well as synthesis of genetic material and gene product (Schmid, 1963; Monesi, 1965). Mukherjee and Sinha (1964) have shown evidence for a random inactivation of one of the two X's with the use of heteromorphic chromosomes of mule.

A search for a similar cytological basis has long been made in *Drosophila*. It was recorded by Dobzhansky (1957) that the single X-chromosome in male

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salivary gland cells of *Drosophila* enlarges to a size almost as large as the two conjugated X's in the female. He has shown that in the F_1 hybrids of *D. tropicalis* x *D. insularis* the arms of the salivary gland chromosomes remain completely asynapsed, and all autosomal arms are of equal size but the single X-chromosome in XY hybrids is much wider than any of the individual autosomal arms. He claimed this effect to be a cytological counterpart of dosage compensation.

No direct proof of correlation between the enlargement of the X-chromosome in the male salivary gland and the genetic dosage compensation, however, exists. But the fact that the X-chromosome is involved and that the effect is unique and unambiguous, leads one to believe that there must be a reason behind the enlargement of the X in male salivary glands. It will be our purpose to present certain facts observed by previous workers and by ourselves along this line.

Nucleic acid-protein content and synthetic activity of the X-chromosome and its bearing on dosage compensation:

Rudkin and his collaborators (see Rudkin, 1964) have reported the results of ultraviolet (257 mμ) and visible light (at 546 mμ following Feulgen staining) absorption by the X-chromosomes in salivary glands of male and female *Drosophila melanogaster*. No difference in the total nucleic acid or DNA or protein content has been observed between the individual X's in the female and the single X-chromosome of the male, except for certain simpler proteins containing little or no cyclic amino acids (peak absorption at 231 mμ). Rudkin (1964) has emphasized that these cytological manifestations of X-linked parts of the genome in *Drosophila* may be cytological counterparts of the genetic dosage compensation and suggested further that the single X of the male might be more active than the individual X's of the female. This postulate has recently been refuted by Muller and Kaplan (1966), although Mukherjee and Beermann (1965) had already demonstrated that the single X in the salivary gland of male *Drosophila melanogaster* synthesizes twice as much RNA as the individual X-chromosomes of the female. On the basis of these findings, Mukherjee (1966) has suggested that (*a*) this enhanced rate of synthesis of RNA on the single X of the male which approaches very close to that in the two X's of the female, may be manifestation of dosage compensation at the molecular and chromosomal levels. In other words, dosage compensation in *Drosophila* acts by a process of regulatory synthesis of gene product at the level of information transfer from DNA to RNA, and it follows, therefore, that it may act by an enhancement in the rate of synthesis in the male rather than by repression in the female. (*b*) Finally, the enlargement of the male X-chromosome may be a result of accumulation of nucleic acids or nucleoprotein material.

The present authors have been examining in detail the possible qualitative and/or quantitative difference in the pattern of incorporation of H^3-Uridine in the X-chromosome of the two sexes in *D. melanogaster* and *D. hydei*. In his earlier work Mukherjee (1966) has shown that there is an accurate concordance between

Table 1 *Mean of the absolute number of grains over the fraction and the whole of X— and 4-Chromosomes and the mean of the ratios obtained by comparing X-chromosome and autosome in* D. hydei

	Mean Number of Grains				Mean Ratio			
	X-fr (12A-20D) (a)	4-fr (85A-94D) (b)	X-whole (1A-20D) (c)	4-whole (73D-94D) (d)	a/b	c/d	a/d	c/b
I. Low—Labelled Group								
Female	125·46 (11)	102·82 (15)	246·90 (15)	210·57 (15)	1·22	1·12	0·55	2·55
Male	141·20 (18)	130·30 (17)	300·50 (18)	261·40 (18)	1·07	1·11	0·54	2·20
II. High—Labelled Group								
Female	367·06 (11)	347·96 (11)	783·44 (10)	707·59 (10)	1·14	1·08	0·52	2·28
Male	271·50 (30)	278·22 (30)	535·50 (28)	579·70 (22)	1·00	1·03	0·46	1·99

Number in parenthesis denotes the number of nuclei observed.

the number of grains (H^3) on the single X-chromosome in the male and that on the two conjugated X-chromosomes in the female (measured in both cases in relation to the number on a standard conjugated autosome). Recent works carried out in this laboratory further confirm this observation. Figs. 1 and 2 represent the two labelling patterns in the X-chromosomes of *D. hydei*. It is apparent that patterns of incorporation of H^3-Uridine are identical in the two sexes, irrespective of low or high labelling.

The pattern of incorporation may be outlined as follows: the nucleolus and typical puffs in all cases show the highest incorporation. The less stained or diffusely stained bands (and interbands) have a second degree of labelling. The heavily stained bands e.g., 2A1, 4B2, 7A1 and 18B3-4 of the X-chromosome do not show any incorporation or very negligibly so in most cases, irrespective of sexes. Table 1 shows the results of grain counts on the X and a standard autosome. It is apparent upon the comparison of the ratios in the last four columns that for both high and low labelled groups the relative proportions of grains on the single male X and on the conjugated double female X's are similar. These results show that in both *melanogaster* and *hydei* the single X-chromosome in the male works twice as hard as the individual X's of the female in its transcribing acti- vity. It would, however, be too early at present to establish its relationship with the genetic dosage compensation. But inspite of Muller and Kaplan's (1966) statements, the fact that the single X of the male incorporates almost

Fig. 1. Pattern of H^3-Uridine incorporation in the X-chromosome and autosomes in (*a*) female and (*b*) male salivary gland of *D. hydei*—high labelled group.

twice as much tritium as the individual X's of the female cannot be overlooked, and it may confidently be stated, "the single X of the male works twice as hard ast he individual female X's."

Very recently, Berendes (1966) has reported the relative rate of synthesis of DNA by the X-chromosomes in the female and that in the male salivary gland of *D. hydei*. He has shown that among cases with discontinuously labelled pattern, a striking difference is observed in males between the X-chromosome and the autosomes. Furthermore, 55% of the heavy continuously labelled male nuclei showed heavy discontinuously labelled X-chromosome, an effect that has never been observed in the female. The fact that the labelling pattern of the asynapsed X-chromosome in the female corresponds more closely with that of the conjugated X's of female than with that of the male X-chromosome, further shows that this difference is not the consequence of the X-chromosome of male being single. Berendes (1966) has concluded that the period needed for complete replication is shorter in the single male X than in the double female X-chromosomes, and has suggested that the enlarged configuration of the X-chromosome in the male may be due to the fact that the DNA is loosely coiled in the male. This suggestion appears to fit in with the enhanced rate of synthesis of RNA in the male X-chromosome as observed by Mukherjee and Beermann (1965) and by the present investigators, since uncoiling or a loose coiling of DNA may necessarily be a possible condition in initiating an enhanced rate of synthesis of RNA.

Puffing activity in the X-chromosome

A comparison of puffing activity in the X-chromosomes of male and female larval salivary glands of *D. hydei* shows absence of any striking difference in the puffing activity in the two sexes (Berendes, 1965). Unpublished work of A.K. Dutta Gupta in this laboratory on the puffing activity in the X-chromosome of *D. ananassae*, on the contrary, shows that at least for certain segments,

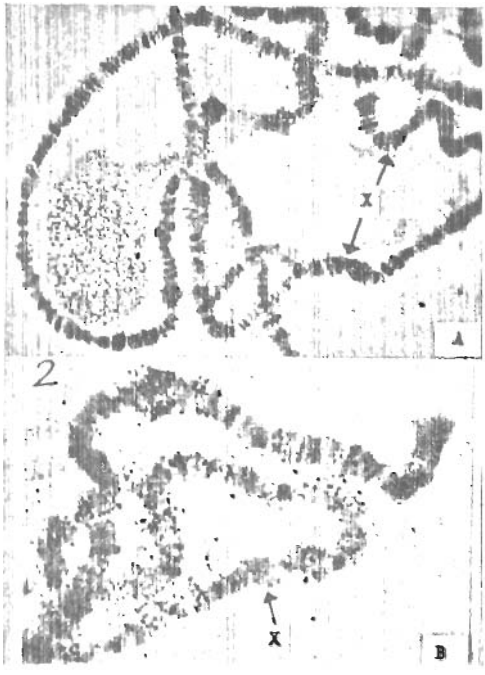

Fig. 2. Pattern of H^3-Uridine incorporation in the X-chromosome and autosomes in (*a*) female and (*b*) male salivary gland of *D. hydei*—low labelled group.

the activity pattern appears to be different in the two sexes at a specific stage of development. However, this example may be an exception rather than a general rule, specially since activities of these segments are known to be influenced by changes in configuration of a neighbouring heterochromatic region (preliminary report, Mukherjee and Dutta Gupta, 1968).

It has been shown that dicyandiamide, a cyanoguanidine, which is known to enhance the rate of protein synthesis (Steinman *et al.*, 1965) initiates a change in the puffing activity in *D. melanogaster* (Mukherjee, 1968) as well as in *D. hydei*. However, the normal puffing pattern, and that induced by dicyandiamide is more or less similar in the two sexes. It appears, therefore, that there may be certain precise differences in the molecular organisation of the X-chromosomes of the male and female which seem to react differently in order to compensate for the dosage difference. If this is the case, then it would imply that this behaviour may reflect preparatory events at the chromosomal level for what would actually happen in the final phenotypic level of genetic dosage compensation.

Width of the X-chromosome in the two sexes:

Rudkin (1964) has shown that the area of a segment of the single X in the male is significantly larger than a corresponding segment of an asynapsed X in the female, and since the lengths in the two are identical it is apparent that the difference should be due to a difference in the width of the X-chromosome. Muller and Kaplan (1966) do not consider that the male's single X enlarges to almost the width of the double X's of the female salivary gland. They have questioned the validity of the measurements of the area of the short regions of X-chromosomes of the male and female in Rudkin's report. It may be pointed out in this context that the method adopted by Muller and Kaplan to compare *their* data with Rudkin's is presumably different from that adopted by the latter.

Berendes (1966) on the other hand shows that the single X of male is indeed enlarged. Although no details of the measurements have been available from this report, he has mentioned that the ratio of the diameter of the conjugated double X of female to single X of male to asynapsed single X of female in the salivary glands of *D. hydei* is 1·2: 1·0: 0·7. In an independent experiment carried out in this laboratory the width of the X-chromosome in relation to a standard autosome in male and female larval salivary glands of *D. melanogaster* has been measured and the above ratios have been found to be in the order of 1·00: 0·91: 0·68.

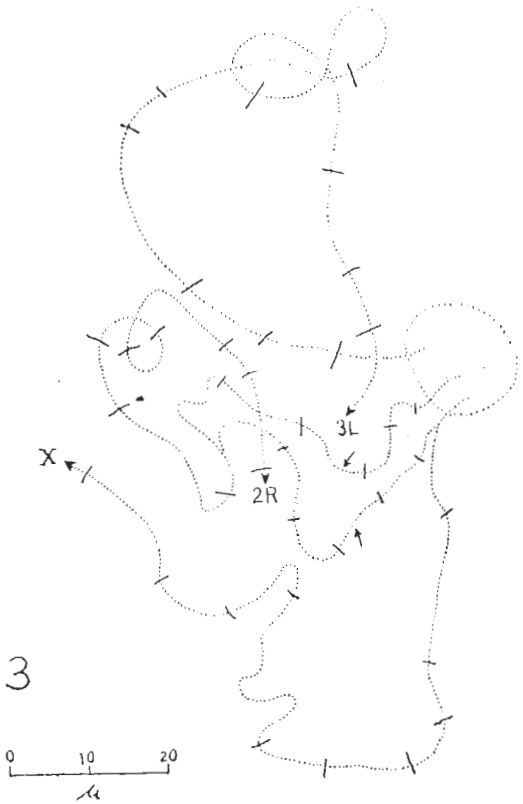

Fig. 3. An orientation map of the X, 2R and 3L chromosomes in the salivary gland of *D. melanogaster*, drawn under camera lucida, showing the method used in the measurement of chromosomal width in X-irradiated series. The crossbars on the chromosomes indicate the width of the individual regions measured. The two arrows on the proximal region of the 2R show the asynapsed portion of this autosomal arm.

In this series of experiments, the linear outline of the X-chromosome and one arm of a particular long autosome (3L in this case) were drawn using a Camera Lucida along with certain bands, deliberately avoiding the puffs and constrictions (fig. 3). The length of the diameter of the band was then measured from the drawing with the use of a vernier-attached Slide Calliper. The results of these measurements are shown as ratios of width of the X-chromosome to that of 3L (table 2).

Although there is some discrepancy between our measurements and those of Berendes (1966), in a general way both observations indicate that the X-chromosome is in fact enlarged in the male salivary gland as compared to the individual X-chromosome in the female. This difference may be attributed to a different degree of polytenization of the chromosomes in the two species. The difference due to the different techniques of measurement may not however be excluded. The difference between Berendes' and our measurements and those of Muller and Kaplan (1966) may be mainly due to the latter reason. A

detailed analysis of the width of the X-chromosome in the two sexes under experimental conditions further reveals that the width of the male X is greatly sensitive to certain experimental conditions. The effect of X-irradiation only has been presented here.

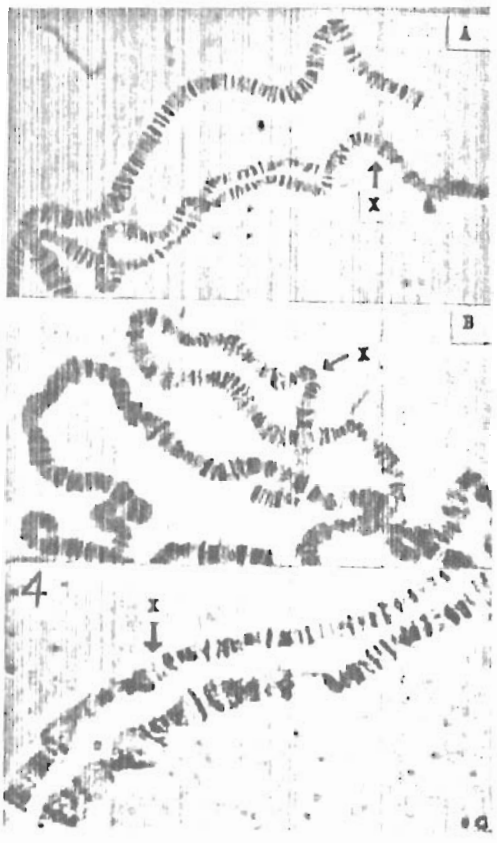

Fig. 4. The X-chromosome and an autosomal arm showing the relative width in (*a*) control female, (*b*) control male and (*c*) irradiated male larval salivary gland of *D. melanogaster*. In (*c*) only a portion of the chromosomes is being shown.

X-irradiation of the whole larvae with 500 to 5000r shows that this treatment selectively reduces the width of the male X (table 2). The effect is not all-or-none. Figs. 4(a), (b) and (c) present the relative size of the X and autosomes in control female, control male and X-irradiated male (extreme case), respectively. As evident the extreme cases of reduction are more frequent in the higher doses than in the lower (table 2). The effect is reversible in 500r and perhaps also in higher doses (fig. 5). A comparison has also been made on the effect of X-irradiation on asynapsed chromosomes with that on the single X of the male. The data are presented in table 3 and show that only the difference of the ratios between control male and treated male is significant at 1% level, *t* being equal to 5·4.

These results indicate that whatever may be the factors responsible for the enlargement of single X of male, the material is reasonably labile and susceptible to removal or repression by extraneous agents. The effect may be at the level of DNA configuration itself in the male X, as suggested by Berendes (1966) or at the level of accumulation of certain transcription product or even at the level of nucleoprotein binding.

With these available facts it may be suggested that perhaps the cytological observations presented by Muller and Kaplan (1966) may be analysed in detail. The materials used for their cytological analysis were the X; 4 translocations and comparison of the width was based on the X-to-4th transition. But the 4th chromosome, perhaps being very short or perhaps for some other reasons not

Table 2 *Comparison of the ratios of average width of entire X-and 3L-chromosomes (3L/X) in male and female nuclei with and without X-irradiation*

	Percent of Nuclei in different ranges of Ratios										N	Mean ratio	Value of *t*
	0·71 to 0·80	0·81 to 0·90	0·91 to 1·00	1·01 to 1·10	1·11 to 1·20	1·21 to 1·30	1·31 to 1·40	1·41 to 1·50	1·51 to 1·60	1·61 to 1·70	1·71 to 1·80	1·81 to 1·90			
Control															
Female	—	7	46	40	7	—	—	—	—	—	—	—	59	1·00±0·001	—
Male	—	3	8	42	33	12	2	—	—	—	—	—	60	1·10±0·011	7·3**
500 *R:* 4-5 *hr after*															
Male	—	—	—	7	17	23	37	13	3	—	—	—	30	1·30±0·022	9·3*
1000 *R:* 3-5 *hr after*															
Male	—	—	—	10	7	29	25	18	11	—	—	—	28	1·32±0·03	7·1*
3000 *R:* 3-5 *hr after*															
Male	—	—	—	7	23	27	30	10	3	—	—	—	30	1·28±0·021	8·6*
5000 *R:* 3-5 *hr after*															
Female	—	3	33	54	7	3	—	—	—	—	—	—	30	1·03±0·015	1·8
Male	—	—	—	—	7	37	23	23	7	3	—	—	30	1·35±0·022	11·7*

*=only these *t* values are significant at 1 percent level when compared with the ratio of the corresponding control male.
**=this *t* value is significant at 1 percent level when compared with the ratio of control female.

known is somewhat wider than all chromosomes. It is apparent even from the photographs presented in their paper that the measurement would give a different result if the width of the X were compared with any long arm of the two larger autosomes, viz. 2L, 2R, 3L or 3R. In a report Schultz (1965) has discussed in detail the significance of X-chromosomal inflation in male salivary glands of *Drosophila* and he has emphasized the hyperactivity of male X. He has further stated that "by-and-large this hyperaction in the male, as judged by the inflation of the X-fragments (in X-autosome translocations), is present in a variety of loci in the X-chromosome, just as the dosage compensation studied genetically by Muller is scattered through the X". Recently, Stern (1968) in his discussion on different mechanisms of dosage compensation in mammals and *Drosophila*, has also stressed on the operation of dosage compensation through male in *Drosophila*.

General Discussion:

These facts may be

Table 3 *Comparison of the ratios of chromosomal width in conjugated and asynapsed condition with and without X-irradiation*

	Number of nuclei in different ranges of ratios												N	Mean Average Ratio	Value of *t*
	0·51 to 0·70	0·71 to 0·80	0·81 to 0·90	0·91 to 1·00	1·01 to 1·10	1·11 to 1·20	1·21 to 1·30	1·31 to 1·40	1·41 to 1·50	1·51 to 1·60	1·61 to 1·70	1·71 to above			
I. 1*A*/1*X*															
A. Male															
Control	10	6	3	—	—	—	—	—	—	—	—	—	19	0·69±0·023	
Treated	2	15	12	4	1	—	—	—	—	—	—	—	34	0·82±0·014	5·4*
B. Female															
Control	—	—	—	1	2	—	—	—	—	—	—	—	3	1·02±0·015	
Treated	—	—	1	1	2	—	—	—	—	—	—	—	4	0·97±0·052	0·25
II. 2*A*/1*A*															
A. Male															
Control	—	—	—	—	—	—	2	1	1	5	3	4	16	1·61±0·061	
Treated	—	—	—	—	—	—	—	4	4	12	7	2	29	1·56±0·020	1·0
B. Female															
Control	—	—	—	—	—	—	—	2	—	2	3	1	8	1·54±0·048	
Treated	—	—	—	—	—	—	—	1	4	4	2	5	16	1·59±0·041	0·73
III. 2*A*/1*X*															
A. Female															
Control	—	—	—	—	—	1	—	2	1	—	4	—	8	1·48±0·064	
Treated	—	—	—	—	—	—	2	2	2	4	4	3	17	1·55±0·040	0·92
IV. 2*X*/1*X*															
A. Female															
Control	—	—	—	—	—	—	—	2	—	3	1	—	6	1·51±0·016	
Treated	—	—	—	—	—	2	—	—	5	4	4	—	15	1·49±0·030	0·3

*=only this *t* value is significant at 1 percent level when compared with the ratio of the corresponding control male.

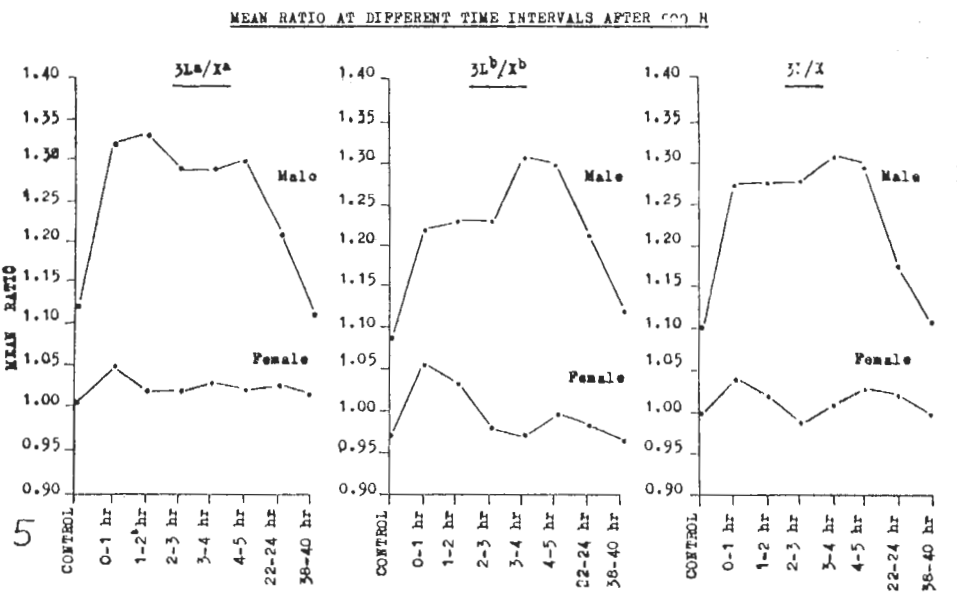

Fig. 5. Curves showing mean ratio (ordinate) in control male and female and at different time intervals after 500 r (abscissa) in male and female larvae of *D. melanogaster*. $3L^a/X^a$ represents ratio of average width of proximal part of 3L (band 80 to 71) to that of proximal part of X (band 20 to 11); $3L^b/X^b$ represents ratio of average width of distal part of 3L (band 70 to 61) to that of distal part of X (band 10 to 1); and 3L/X represents ratio of average width of entire 3L to that of entire X.

critically analysed in the light of the relationship that may exist in the chain of reactions from DNA in the chromosome to the final product. Genetic analysis has clearly demonstrated that for certain sexlinked characters, the gene expression is identical in the two sexes and it is not dependent on the development of the sex (Muller, 1932; Stern, 1929; Lieb, 1942, quoted in Cock, 1964). An interesting argument by Cock (1960) may be discussed in this connection. While Muller's theory implies that dosage compensation operates by a cancellation effect of certain plus and minus modifiers in the female, Dobzhansky (1957) suggested that the enlargement of the male X-chromosome in the salivary gland is a cytological counterpart of dosage compensation and is a result of accumulation of some products of gene activity. Cock (1964) has attempted to reconcile the apparently opposite views by assuming that "it is the absence of the X-enlargement in the female which represents the compensation phenomenon". It is possible that the compensation may have its respective assignments in both sexes but there is no reason to believe that it can not operate through males.

With reference to the problem whether enlargement of the X in the male salivary gland can be considered as cytological counterpart of dosage compensation, it is worth mentioning that the structural and functional morphology of the

salivary gland X-chromosome in the male itself suggests that there must be certain reasons for its peculiar characteristics, as for example (*a*) enlargement of X, (*b*) similarity in puffing activity pattern in the male and female X's and (*c*) similarity in the rate of RNA synthesis in the X-chromosome of the two sexes. To these may be added another fact, though not well understood, that the X-chromosome in the salivary gland of male *Drosophila* often spreads out even when the remaining chromosomal arms remain entangled in a mass.

The fact that this enlargement may be artificially changed and that there is a remarkable increase in the rate of RNA synthesis, indicates that whatever may be the actual significance of enlargement it is of a kind of transitory arrangement perhaps necessary to augment the compensation effect.

A Working Hypothesis:

It may be proposed at this stage as a working model that the operative mechanism *has its own assignment in both sexes* but its cytological expression in the male salivary gland as observed in the enlargement of the X is a manifestation of an overall higher genetic activity, induced by the absence of appropriate number of plus and minus modifiers. Whether the enlargement is due to nucleic acids, protein or nucleoproteins or any other chemicals remains unsolved. Further work along this line perhaps may resolve the complicated phenomenon and its bearing on dosage compensation. It is, however, interesting to observe that under the influence of refined knowledge of genetics during the last decade and its rapid amalgamation with cytology, cell physiology, molecular biology resulting in the formation of a new alloy—the gene physiology—the study of dosage compensation has taken a new turn and the chapter is far from being closed (Stern, 1960).

Summary

In *Drosophila* the single X-chromosome in male salivary glands appears to compensate the dosage of sexlinked genes by an enlargement to nearly the size of, and performing as much transcriptive activity as, the paired X-chromosomes of the female. The pattern of RNA synthesis in the X-chromosomes is highly concordant in the two sexes, in both species, *melanogaster* and *hydei*, in spite of their having different X-chromosome constitution. X-irradiation (0·5 to 5 Kr) induces considerable reduction of the width of the male X, in a manner not conforming with an all-or-none mechanism (average ratio of width,—female's 2X: male's 1X = 1·10 in control, 1·23 to 1·35 in treated; significant at 1% level). The reduction is reversible and may involve the whole or a part of the X. Furthermore, a differential degree of puffing activity has *not* been observed in the X-chromosomes in the two sexes in *D. hydei* (except some minor plus or minus activity difference), under both normal and experimentally induced conditions.

It appears that the *modus operandi* of dosage compensation underlie a repressible (as in females) or an inducible (as in males) control by one or more groups of localized regulatory loci, each of which may have an assignment on a specific set of genes (piece-meal device of Muller and Kaplan). Their controlling functions are realized at the chromosomal level only in the male, by a stepwise enlargement of the X and an enhanced rate of RNA synthesis.

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B. Erich Wolf (Berlin): Did you consider that there are differences between X-chromosome and autosomes in *Drosophila* by allocycly of the former, often to be observed in the male ? I found the X-chromosome of *Drosophila melanogaster* male sometimes small, sometimes of the same thickness as

autosomes and like the X-chromosome in the female. In the latter case it appears to be less stretched than in cases with X-chromosomes. Have you measured length and breadth of the X-chromosomes in the different cases ?

A. S. Mukherjee (Calcutta): It is true that you get certain degree of variation in the width of the X-chromosome in *Drosophila* males, but we have considered the problem of "stretching" which may influence the width. For avoiding error we, therefore, have also made measurements of width of the X and an autosome segment having a constant length—in unstretched condition. Thus, although there still remains the possibility of localised stretching, we have attempted to avoid measurements from chromosomes having an overall stretching.

A. P. Jha (Bhagalpur): Could you recognise any main gene with switch mechanism attributed to modifiers, inhibitors, etc. ?

A. S. Mukherjee (Calcutta): Yes, that is the basis of recognizing dosage compensation which has been so nicely demonstrated by Muller and his collaborators using different parts of the X. And it is obvious that compensatory function is distributed throughout the whole of the X. Existence of dosage compensators is further demonstrable by the effect of a sex-linked mutant gene, *zeste* (*z*), which itself does not show dosage compensation. In this case, a normal allele of *white* (*w*) acts as modifier of *z* and the non-compensatory sex-dimorphism is influenced by the dosage of the *w* allele.

R. B. Singh (Hissar): Has the sex determining mechanism in different types of organisms something to do with the mode of dosage compensation ?

A. S. Mukherjee: No, Sex-determination has no direct bearing on the dosage compensation and the genes responsible for sex-determination are independent of the compensators.

S. K. Roy (Varanasi): Do the puffing patterns have any genetical or metabolic significance for the individual ?

A. S. Mukherjee: Yes. You will find answer to your question in the discussions by Pavan, Wolf and others in this symposium. I may simply add that puffs are in fact the genetically active sites and certain puffs are indeed of metabolic significance.