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Chromosomal basis of dosage compensation in *Drosophila*

I. Cellular autonomy of hyperactivity of the male *X*-chromosome in salivary glands and sex differentiation*

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1. INTRODUCTION

The enlargement of the *X*-chromosome in larval salivary glands of male *Drosophila* has been noted by many workers and this has been considered to be the chromosomal manifestation of dosage compensation (Offermann, 1936; Aronson, Rudkin & Schultz, 1954; Dobzhansky, 1957; Rudkin, 1964; Schultz, 1965; Mukherjee & Beermann, 1965; Mukherjee, 1966; Mukherjee, Lakhotia & Chatterjee, 1968; Stern, 1968). Mukherjee and his collaborators have shown that the male *X*-chromosome in larval salivary glands of *Drosophila* synthesizes RNA at a rate equal to that of the paired *X*'s of the female. It has also been observed that the enlargement of the male *X* in *D. melanogaster* is reversible under the action of X-rays and of certain chemicals known to inhibit chromosomal RNA synthesis (Lakhotia, unpublished). This and other histochemical evidence, e.g. differences in the DNA replication pattern of the *X*-chromosome in the male and female (Berendes, 1966; Rodman, 1968) support the surmise that *X*-chromosomal enlargement accompanied by the increased rate of RNA synthesis in male larval salivary glands of *Drosophila* may be considered as the chromosomal basis of dosage compensation (see Discussion).

A major controversy over the mechanism of dosage compensation in *Drosophila* still remains. While Muller (1950) and others (see Stern, 1960) believed in the independence of the dosage compensation mechanism and the sex-differentiating system, Goldschmidt (1954, 1955) considered differences in sex-physiology of male and female to be responsible for dosage compensation. Recently, Komma (1966) and Lee (1968) have reported their findings which have been interpreted as evidence that support Goldschmidt's idea. On the other hand, Smith & Lucchesi (1968) have concluded on the basis of their spectrophotometric analysis of eye colour mutants that differences in sex-physiology do not mediate dosage compensation in *Drosophila*.

In this series of investigation, further evidence has been sought to establish that the hyperactivity of the male *X*-chromosome, expressed by its enlargement and enhanced rate of RNA synthesis, may be considered as a cytological counterpart

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of dosage compensation, and to find out whether this hyperactivity is dependent on the sex of the individual—as required by Goldschmidt's theory of dosage compensation in *Drosophila*. For this purpose, the unstable ring-*X*-chromosome, which is frequently eliminated from certain cells during division, was utilized to produce *XX*/*XO* mosaic salivary glands. This provides us with a unique opportunity to demonstrate the role of sex-physiology on the hyperactivity of the male *X*-chromosome in *Drosophila* salivary glands and thereby to understand the possible relation between the hyperactivity, dosage compensation and sex-differentiation. In the present communication, comparative observations on the morphology and pattern of RNA synthesis of the *X*-chromosome in such mosaic glands developing in a sexually female background (i.e. otherwise genotypically *XX*) will be presented.

2. MATERIAL AND METHODS

The following stocks of *D. melanogaster* were used for these experiments (for details of abbreviations, see Bridges & Brehme, 1944): (1) In (X^{c2})w^{vc}/In(1)dl-49, $ywlz^{s}$ female; In (1)dl-49, $ywlz^{s}/sc^{8}.Y$ male, and (2) *ywct* male and female. Five to seven days old virgin ring-*X* females from the stock (no. 1 above) were mated to *ywct* males and mature third-instar larvae (F_1) sarificed for salivary gland chromosome analysis. While the non-ring-*X* female larvae obtained in the F_1 would be homozygous for both *y* and *w* and therefore should have yellow mouthparts (unless suppressed by the floating $sc^{8}.Y$) and colourless Malpighian tubules, the ring-*X* female larvae would be heterozygous for both *y* and *w* and should have black mouthparts and bright yellow Malpighian tubules. Only those larvae which could be distinctly identified as females by their gonads and had mouthparts black and Malpighian tubules yellow were taken for salivary gland preparation. The presence of ring-*X* in their glands was verified cytologically. For morphological observations squash preparations were made by the usual method of aceto-carmine–aceto-orcein staining and squashing in lacto-aceto-orcein. The parental stocks as well as the larvae were raised on standard cornmeal-agar *Drosophila* food at 24 ± 1 °C.

Each preparation, containing only one pair of glands from a single larva, was scanned for the presence of *XO* nuclei which were identified by the absence of the ring-*X* and presence of only one rod-*X*. The number of *XO* and *XX* nuclei in each mosaic gland was scored only from mature nuclei since they alone could be observed with definite clarity. Since the ring-*X* stock carried a floating $sc^{8}.Y$, the mosaics observed could be either *XX*/*XO* or *XXY*/*XY*; in both cases the single-*X*-bearing nuclei will be referred to as *XO*. In one of the mosaic glands (larva no. 5, Table 1) instead of the paternal non-inversion rod-*X*-chromosome, In dl-49-*X*-chromosome was present. This probably had resulted from non-disjunction in the parental female. Nuclei from this pair of mosaic glands have also been considered together with those from other *XX*/*XO* mosaics.

Preparations which contained both *XX* and *XO* nuclei were selected for measurement of the width of chromosomes. The width of the *X*-chromosome and

Table 1

A. Mean absolute chromosomal widths in different mosaic glands*

Larva no.	*XX* nuclei, mean absolute width					*XO* nuclei, mean absolute width			*XO* nuclei in gland (%)
	XX	X^{ro}	X^{rl}	$3L^{t}$	N^{t}	X^{m}	$3L^{m}$	N^{m}	
1	7·42 ± 0·29	5·37 ± 0·12	4·99 ± 0·22	7·45 ± 0·35	5	6·37 ± 0·00	7·13 ± 0·00	1	0·5
2	6·91 ± 0·23	4·53 ± 0·24	4·63 ± 0·17	6·95 ± 0·29	5	6·36 ± 0·22	6·93 ± 0·28	5	96·0
3	6·42 ± 0·34	4·73 ± 0·16	4·18 ± 0·15	6·56 ± 0·30	4	6·17 ± 0·30	6·42 ± 0·25	9	17·0
4	7·09 ± 0·26	5·12 ± 0·46	4·86 ± 0·15	6·90 ± 0·26	5	6·15 ± 0·26	6·76 ± 0·33	5	7·0
5	6·18 ± 0·24	4·03 ± 0·13	4·26 ± 0·19	6·06 ± 0·28	7	5·44 ± 0·11	6·03 ± 0·17	8	8·0
6	5·38 ± 0·19	3·99 ± 0·12	3·74 ± 0·13	5·81 ± 0·18	6	5·66 ± 0·15	6·01 ± 0·14	7	49·0
Av. mean	6·57	4·63	4·44	6·62	32	6·02	6·55	35	

B. Mean ratios of widths of chromosomes in different mosaic glands

Larva no.	Mean ratios							
	$3L^{t}/XX$	$3L^{m}/X^{m}$	$3L^{t}/3L^{m}$	XX/X^{ro-rl}	XX/X^{m}	X^{m}/X^{ro-rl}	$X^{ro-rl}/\frac{1}{2}XX$	$X^{m}/\frac{1}{2}XX$
1	1·00	1·12	1·04	1·43	1·16	1·23	1·37	1·72
2	1·00	1·09	1·00	1·52	1·09	1·39	1·31	1·84
3	1·02	1·04	1·02	1·44	1·04	1·40	1·35	1·92
4	0·97	1·10	1·04	1·47	1·16	1·23	1·40	1·73
5	0·98	1·10	1·00	1·49	1·13	1·31	1·34	1·76
6	1·08	1·06	0·97	1·40	0·95	1·46	1·44	2·10
Av. mean	1·01	1·09	1·01	1·45	1·09	1·34	1·37	1·84

* The values represent chromosomal width as measured from camera-lucida drawings by the use of a vernier-attached slide-calliper and measured in millimetres (for details, see Mukherjee *et al.* 1968).

the left arm of the 3rd chromosome (3L) was measured from camera lucida drawings using a vernier-attached slide-calliper by the method described previously (Mukherjee *et al.* 1968). The width was measured from *XX* and *XO* nuclei selected at random—except one restriction that they had good and uniform spreading of chromosomes. In each *XX* nucleus the width of the paired part of ring-/rod-*X*'s (*XX*), unpaired part of ring-*X* (X^{ri}), unpaired part of rod-*X* (X^{ro}) and the paired 3*L* ($3L^{t}$), and in each *XO* nucleus the width of the single rod-*X* (X^{m}) and the paired 3*L* ($3L^{m}$) was measured. All camera-lucida drawings were made under the same magnification (× 95, × 10), and since the *XX* and *XO* nuclei were selected at random, and therefore the samples should be more or less of similar polytenic class, the absolute widths of the *X*-chromosome in the two types of nuclei are directly comparable. This can be further checked by comparing the absolute widths of the 3*L* in *XX* and *XO* nuclei, respectively, from any one pair of mosaic glands. For obtaining the width of an asynapsed *X* of *XX* nuclei, average of widths of the rod- and ring-*X*'s (X^{ro-ri}) has been used.

For studying the pattern of RNA synthesis, each pair of glands from a single larva as above were incubated in 0·02 ml of *Drosophila* ringer (pH 7·0, Berendes, Van Breugel & Holt, 1965) containing 2 μC of [^{3}H]uridine (sp. act. 3·6 C/mM) for 10 min and were then immediately fixed in aceto-alcohol. The preparations were stained as above and squashed in 50 % acetic acid. The coverslips were removed by floating them off in a 1:1 mixture of 50 % acetic acid and 50 % ethyl alcohol. Both slides and coverslips were covered with Kodak AR 10 stripping film* following the usual procedure. The coverslips were mounted on a clean slide by means of a glass adhesive with material side up prior to covering them with the film. After 20 days of exposure in the dark at 4–8 °C the preparations were developed in Kodak D 19*b* for 10 min at 10 °C and fixed in Kodak Rapid Acid Fixer at the same temperature (this low temperature for developing and fixing was employed because at a higher temperature the films tended to slip off the preparations). After washing, the autoradiograms were stained with 0·2 % toluidine blue in 30 % alcohol, dehydrated and mounted.

3. RESULTS

A. Morphology of X*-chromosome in* XX/XO *mosaic salivary glands*

Width of the chromosomes was measured from *XX* and *XO* nuclei of mosaic salivary glands of six larvae. Complete *XO* or complete *XX* salivary glands were also observed, but no width measurement was made from such nuclei since experience from pilot experiments has shown that the morphology of the *X*-chromosome in such nuclei is similar to that in regular *XY* male and *XX* female larval salivary glands, respectively. The *XO* nuclei in any mosaic gland were at once apparent by the characteristic pale staining and enlargement of their single *X*-chromosome as found in regular *XY* nuclei of normal male salivary glands. This

* Films were kindly given by Drs W. Beermann, C. Pelling and C. Stern, which we gratefully acknowledge.

fact becomes evident from the photomicrographs presented in Figs. 1–4. In each of these figures a part of the squashed mosaic glands has been shown under low magnification and an XX and an XO nucleus from the same field has been presented in higher magnification. The data presented in Table 1A and B make it clear that the width of unpaired X's of female nuclei is not half that of the paired X's. A similar situation exists for autosomes. As shown earlier (Mukherjee, Lakhotia & Chatterjee, 1968), the ratio of width of a paired autosome to that of an unpaired autosome is in the range of 1·5–1·6 but not 2·0. Thus the unpaired condition of a chromosome by itself leads to a width greater than half of the paired chromosome. But the single male X is more enlarged and this cannot be explained by its unpaired condition. Thus, the average ratio of the mean width of an unpaired female X to half of the width of paired X's ($X^{ro-r1}/\frac{1}{2}XX$) is 1·37, while the average ratio of the width of male's single X to half of the width of paired X's of female ($X^{m}/\frac{1}{2}XX$) is 1·84, a value closer to 2·0 (Table 1B). Furthermore, the average ratio of width of paired X's to asynapsed $1X$ in female nuclei (XX/X^{ro-r1}) is 1·45 and the average ratio of the width of paired X's of female to the single X of male nuclei in the same individual (XX/X^{m}) is only 1·09. This direct comparison of the absolute chromosomal widths in XX and XO nuclei may be considered to be valid, since the average width of the $3L$ in XX and XO nuclei of a particular pair of glands is more or less similar (average $3L^{f}/3L^{m}$ ratio being 1·01, column 3 in Table 1B) and since the width of the X in a particular nucleus has been found to be positively correlated with that of the $3L$ ($r = +0{\cdot}7$ to $+0{\cdot}8$; $N = 32$–35). The average ratio of the width of the single X of XO nuclei to the width of the unpaired X's of XX nuclei (X^{m}/X^{ro-r1}) is not 1·0 (which would have been the case if there were no enlargement of the single X in male nuclei), but it is 1·34 instead, a value closer to 1·45 obtained for XX/X^{ro-r1} ratio above. These facts suggest that the single X-chromosome in male nuclei is definitely enlarged and wider than individual X's and nearer to the width of the two paired X's in female nuclei.

Table 2 shows the frequency distribution of the ratios in mosaic glands and in the normal male and female. A comparison of the new ratios in mosaic glands with those in normal male and female shows that neither the mean ratios nor the frequency distributions of these ratios in the two groups are significantly different from each other.

The important point that emerges from these observations is that the enlargement of the single X in male salivary glands is cell-autonomous and that this autonomy is expressed fully and clearly whenever a $2A:1X$ balance is realized, irrespective of the sex of the individual. The last point is specially notable from these data since all the XO nuclei measured are from individuals that were female by their gonads and in addition, in the same tissue (salivary gland), both male and female (XO and XX respectively) cells were functioning normally without any apparent interference. It is clear that the enlargement of the single X in XO nuclei is in no way related to the relative proportion of XO nuclei in a pair of glands. The frequency of XO nuclei in the mosaic glands observed varied from 0·5% (only *one*

Table 2. *Frequency distribution of ratios of chromosomal widths in normal Oregon-R male and female nuclei and in ring-*X *mosaic male and female nuclei**

	% of nuclei in different ranges of ratio												
Chromosomal ratio	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	*N*	Mean ratio	*t* test
I. $3L^{l}/XX$													
Normal ♀	7·0	46·0	40·0	7·0	—	—	—	—	—	—	59	1·00 ± 0·001	$t = 0·53$
Ring-*X* (*XX* nuclei)	12·5	37·5	28·1	18·8	3·1	—	—	—	—	—	32	1·01 ± 0·018	$P > 0·5$
II. $3L^{m}/X^{m}$													
Normal ♂	3·0	8·0	42·0	33·0	12·0	2·0	—	—	—	—	60	1·10 ± 0·011	$t = 0·42$
Ring-*X* (*XO* nuclei)	—	22·8	40·0	31·4	5·8	—	—	—	—	—	35	1·09 ± 0·013	$P > 0·6$
III. $2X/1X$													
Normal ♀	—	—	—	—	—	32·3	—	50·0	16·7	—	6	1·51 ± 0·016	$t = 1·52$
Ring-*X* (*XX* nuclei)	—	—	—	—	12·5	21·9	25·0	28·1	9·4	3·1	32	1·45 ± 0·023	$P > 0·1$
IV. $2A/1X$													
Normal ♀	—	—	—	—	—	25·0	—	25·0	37·5	12·5	8	1·54 ± 0·061	$t = 0·92$
Ring-*X* (*XX* nuclei)	—	—	—	—	15·6	12·5	40·7	15·6	12·5	3·1	32	1·46 ± 0·027	$P > 0·3$

* Data for normal male and normal female from Mukherjee *et al.* (1968).

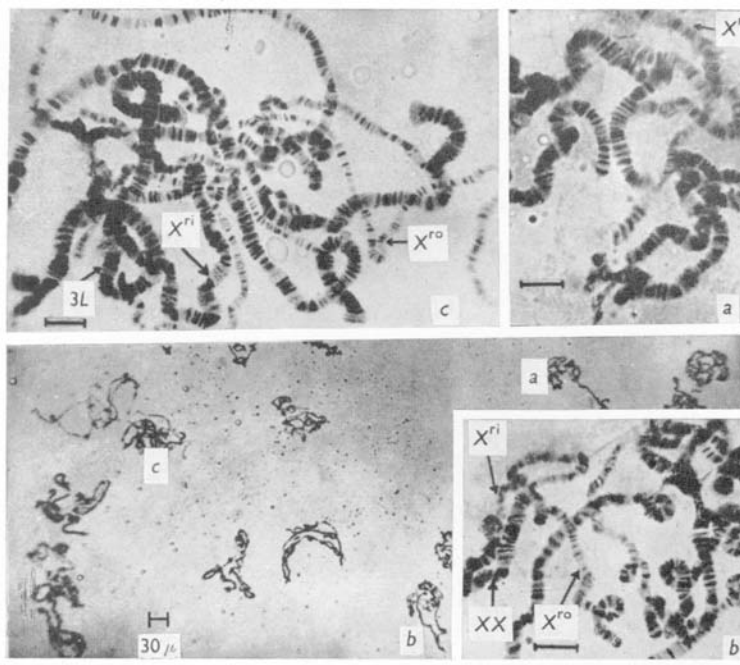

Fig. 1. *XX* and *XO* nuclei from larva no. 1 with 0·5% *XO* nuclei. *a*, The single *XO* nucleus observed in the whole gland; *b*, an *XX* nucleus; and, *c*, one of the 'tetraploid' (or showing chromatid asynapsis) nuclei. Abbreviations in this and subsequent figures are the same as in the text. The scale represents 10 μ in this and all other figures, unless otherwise mentioned.

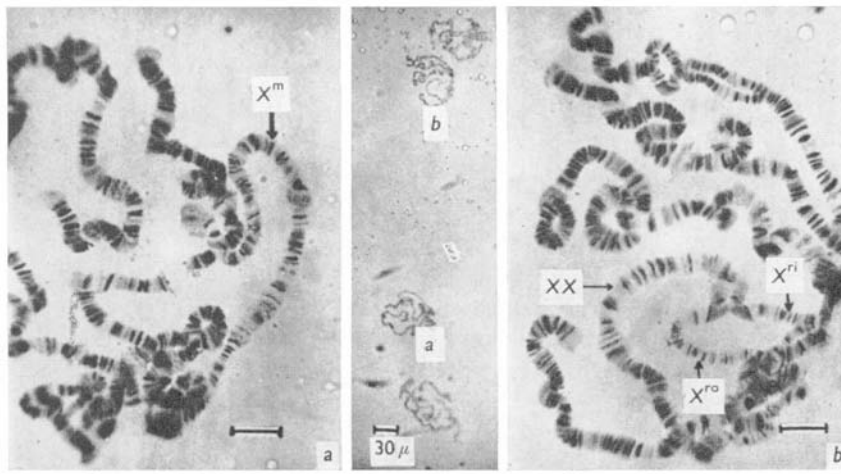

Fig. 2. *XX* and *XO* nuclei from larva no. 2 with 96 % *XO* nuclei. *a*, An *XO* nucleus; *b*, an *XX* nucleus.

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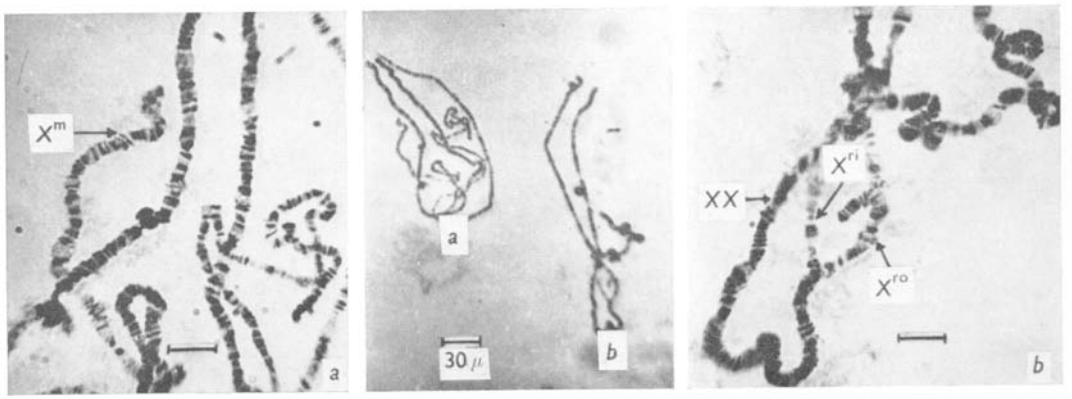

Fig. 3. *XX* and *XO* nuclei from larva no. 3 with 17 % *XO* nuclei. *a*, An *XO* nucleus; *b*, an *XX* nucleus.

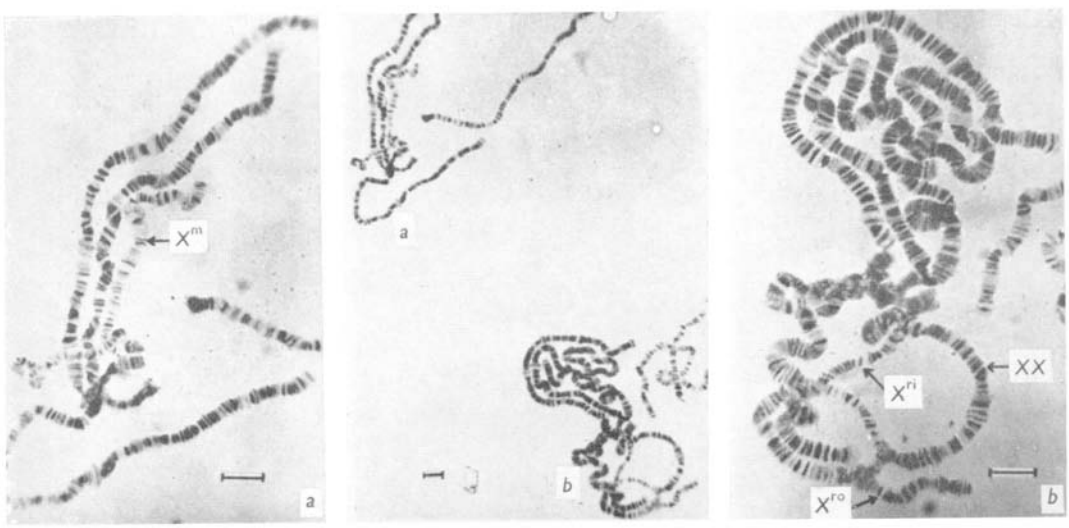

Fig. 4. *XX* and *XO* nuclei from larva no. 5 with 8 % *XO* nuclei. *a*, An *XO* nucleus; *b*, an *XX* nucleus. The rod-*X* in this mosaic carries Indl-49.

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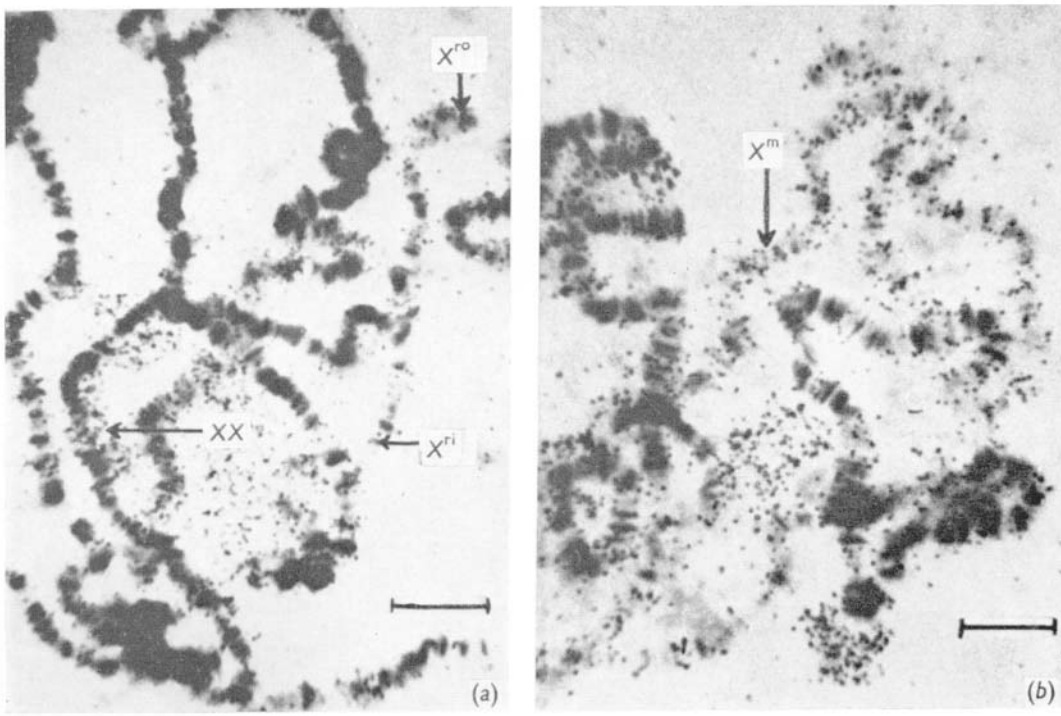

Fig. 5. [^{3}H]Uridine labelling in XX and XO nuclei of a mosaic gland. (*a*) An XX nucleus with paired as well as unpaired parts of the ring- and rod-X's; (*b*) an XO nucleus from the same gland.

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XO nucleus) to 96% (only eight *XX* nuclei) and in every case the autosome to *X*-chromosome ratio is more or less the same (see Table 1, Figs. 1–4). Larva no. 1, with only one *XO* nucleus observed in the whole gland, is worth noting. The *X*-chromosome of this nucleus shows unmistakable enlargement with typical pale staining (Fig. 1*a*) and the $3L^m/X^m$ ratio for this nucleus is 1·12. This value is not much different from the average mean ratio 1·09 since the frequency of ratios in the range 1·11–1·20 has been found to be 31·4% (Table 2). Interestingly, in the same gland two tetraploid nuclei were also present; these had three ring- and one rod-*X*'s. The autosome-to-*X* ratio in these two nuclei has been found to be similar to that in any typical female nucleus (Fig. 1*c*). However, it is also possible that these two nuclei are examples of chromatid asynapsis (Gersh, 1968).

B. RNA synthesis by X-*chromosome in* XX/XO *mosaic glands*

To correlate this autonomous enlargement of the single *X*-chromosome in *XO* nuclei with dosage compensation, the rate of RNA synthesis by the single *X* in *XO* nuclei has been compared with that by the two paired *X*'s in *XX* nuclei of the same mosaic gland. From the autoradiograms, silver grains were counted over two regions on the *X* and 3*L* chromosomes in *XX* and *XO* nuclei of mosaic glands. On the *X*-chromosome the two regions selected were: (*a*) 11A to 20F, and (*b*) 1A to 3B, and on the 3*L*: (*a*) 62A to 68B, and (*b*) 61A to 61F. It is to be noted that in *XX* nuclei selected for grain counting, the region 11A to 20F was always paired and the region 1A to 3B always unpaired, and the grains on this region of the rod-*X* alone were counted; in *XO* nuclei these two regions were obviously single. The two regions on the 3*L* in these *XX* and *XO* nuclei were always paired. This allowed a direct comparison of the rate of RNA synthesis by the single *X* of *XO* nuclei with that of identical regions of paired and unpaired *X*'s of *XX* nuclei.

Table 3 shows the mean number of grains over identical portions of *X* and 3*L* in *XX* and *XO* nuclei from different mosaic glands observed. The grain number on 3*L* serves as a point of reference for the degree of labelling of the *XX* and *XO* nuclei respectively. If there were no increased rate of RNA synthesis by the single *X* of *XO* nuclei, one would expect that under a similar degree of labelling the number of grains on the paired *X*-chromosomes of *XX* nuclei should be twice that on an identical region of the single male *X*, while the number of grains on an unpaired *X* of *XX* nuclei should be equal to that on the single *X* of *XO* nuclei. On the other hand, if there were an enhanced RNA synthesis by the *X* in *XO* nuclei this relation in 2*X* of female to 1*X* of male and 1*X* of female to 1*X* of male nuclei would be reversed. In our experiments the second possibility has been realized. Since the degree of labelling of *XX* and *XO* nuclei in any one pair of mosaic glands is more or less similar, as evident from the labelling of the 3*L* (Table 3), we can directly compare the grain numbers on the *X*-chromosome in *XX* and *XO* nuclei. Such a comparison shows that the average mean number of grains on the region 11A to 20F of the single *X* of *XO* nuclei is equal to that on the identical region of the paired two *X*'s of *XX* nuclei of the same gland (average mean *XX*/*XO* ratio = 0·98). The average mean number of grains on the region

Table 3. *Mean number of grains on* X- *and* 3L-*chromosomes in different* XX/XO *mosaic glands*

	Chromosomal segments in *XX* and *XO* nuclei							
	11A–20F		1A–3B		62A–68B		61A–61F	
Larva no.	*XX**	*XO*	*XX*†	*XO*	*XX*	*XO*	*XX*	*XO*
A	143·5 ± 6·5 (2)	132·8 ± 7·8 (5)	31·4 ± 2·8 (5)	48·6 ± 6·1 (5)	159·0 ± 6·0 (2)	119·4 ± 8·7 (5)	26·8 ± 4·0 (5)	30·2 ± 5·2 (5)
B	131·0 ± 17·3 (4)	132·8 ± 11·3 (5)	27·0 ± 3·1 (4)	49·0 ± 2·9 (4)	125·5 ± 13·8 (4)	123·6 ± 13·6 (5)	28·0 ± 1·6 (4)	30·5 ± 1·0 (4)
C	50·7 ± 3·6 (8)	60·2 ± 5·7 (5)	10·7 ± 1·4 (6)	21·8 ± 2·6 (5)	44·7 ± 5·1 (8)	57·4 ± 6·2 (5)	9·1 ± 1·6 (6)	9·4 ± 1·3 (5)
D	37·8 ± 3·3 (5)	38·8 ± 3·3 (5)	8·2 ± 1·5 (5)	14·0 ± 0·9 (5)	28·8 ± 2·3 (5)	28·4 ± 2·3 (5)	7·2 ± 1·1 (5)	5·8 ± 0·5 (5)
Av. mean ratio	*XX/XO* 0·98		*XX/XO* 0·57		*XX/XO* 1·03		*XX/XO* 1·00	

* The region 11A–20F paired in these *XX* nuclei.
† The region 1A–3B unpaired and single in these *XX* nuclei.
Numbers in parentheses denote the number of nuclei observed.

1A to 3B of the single X of XO nuclei is not equal to but nearly twice that on identical region of an unpaired X of XX nuclei (average mean XX/XO ratio = 0·57). Figure 5 presents labelling pattern in unpaired and paired X's of an XX nucleus, and in the single X of an XO nucleus of mosaic glands. It is clear from these figures and the data presented in Table 3 that the single X in male (XO) nuclei synthesizes RNA equal to that synthesized by the two X's of female (XX) nuclei in the larval salivary glands, a situation similar to that obtained in XY male and XX female individuals.

Like the effect on the morphology of the X-chromosome, the sex of the individual has no effect on the *activity* of the X-chromosome of male or female nuclei. Although the exact number of XO and XX nuclei present in such mosaic salivary glands could not be definitely ascertained due to the possibility of loss of some material during autoradiographic processing, it has been observed that the relative proportion of XO nuclei varied from gland to gland, and this variation in the number of XO nuclei had no effect on the activity of the male X-chromosome.

4. DISCUSSION

The results presented here and those in earlier reports (Offermann, 1936; Aronson *et al.* 1954; Rudkin, 1964; Dobzhansky, 1957; Forward & Kaufmann, 1967; Mukherjee *et al.* 1968) taken together firmly establish that the single X-chromosome in male larval salivary glands in *Drosophila* is considerably more inflated than the individual X's of female. Muller & Kaplan (1966), while not inclined to accept the idea of enlargement of the male X, did not rule out the possibility entirely. They suggested a need for 'actual measurement on *long* portions of the double X that have remained asynapsed, for comparison with corresponding portions of both single and double X's'. The present set of observations essentially fulfils this requirement. The present experimental design, in addition, provided an opportunity for a direct comparison of long paired and unpaired portions of the two X's of the female with the single X of the male in the same pair of glands. Long portions of the female X's, usually including the region from section 1 to 10 of salivary gland chromosome maps of Bridges, remain unpaired due to heterozygosity for the inversion in the ring-X. Actual measurements on the width of the X-chromosome in these nuclei show that while the single unpaired X-chromosomes of female nuclei (XX) are on an average 37% wider than half of the width of the paired two X's, the male's single X in XO nuclei measured by the same standards is 84% wider than the latter, and only 8% narrower than the width of the paired two X's of the female. Thus it is clear that width of the male X-chromosome, although not identical to, very nearly approaches that of the paired two X's of the female. It seems likely that the threshold level of activity is achieved by the male X even without attaining a size identical to that of the paired two X's of the female.

The evidence for a possible correlation between this X-enlargement and dosage compensation comes mainly from studies on metabolic activities of the

X-chromosome in male and female salivary glands (Mukherjee & Beermann, 1965; Mukherjee *et al.* 1968). Furthermore, Berendes (1966) and Rodman (1968) have shown that in *Drosophila* the male *X*-chromosome in the larval salivary glands replicates earlier than the rest of the autosomes. This asynchronous replication of the *X*-chromosome in the male has been interpreted to be due to a loose coiling of DNA within the male *X*, a condition that may be required for a higher genetic activity.

That the male *X*-chromosome in the larval salivary glands of *Drosophila* is genetically more active than other chromosomes is made more than probable by its appearance itself. The whole chromosome by its swelling and pale staining looks like a generalized 'puff', and puffs in giant chromosomes are known to be sites of active RNA synthesis. It has also been observed that agents which interfere with nucleoprotein metabolism, e.g. *X*-rays, benzamide, etc., cause a selective reduction in the width of the male X-chromosome in *D. melanogaster* (Mukherjee *et al.* 1968; and Lakhotia, unpublished). The rate of RNA synthesis in salivary glands, as in other systems, is related to the degree of genetic activity (Beermann, 1964; Pelling, 1964; Berendes, 1967) and dosage compensation is nothing but a regulated expression of *X*-linked genes in the two sexes. Under the circumstances, production of chromosomal RNA at an equal rate by the two *X*'s of the female and one *X* of the male means that they are activated to an identical level and that the phenotypes, which the individual genes on the *X*-chromosome control, are expressed identically in the two sexes because of this hyperactivity of the male *X*.

In the present set of experiments, as in *XX* and *XY* female and male, respectively, the rate of synthesis of the immediate gene product (RNA) by the two *X*'s of *XX* and one *X* of *XO* nuclei present simultaneously in the same pair of glands has been found to be equal. In an independent set of experiments, the expression of *X*-linked eye-colour mutants in *D. melanogaster* in a system similar to that used for the present work is being observed. Preliminary results with the *white* series show that for non-compensated alleles (e.g. w^e, white eosin) the colour of an *XO* eye in an otherwise *XX* female is as light as that in *XY* males while the alleles which are normally compensated (e.g. w^a, white apricot) produce an eye colour in *XO* eyes as dark as in normal *XY* male or in *XX* female eyes (Lakhotia, unpublished). The autonomy of genetic dosage compensation and the cellular autonomy of *X*-enlargement and hyperactivity in male salivary gland nuclei provide strong evidence that the hyperactivity and enlargement of the male *X* is the chromosomal basis of dosage compensation in *Drosophila*.

The theory of *X*-enlargement and *X*-hyperactivity in males as the basis of dosage compensation in *Drosophila* can be reconciled with Muller's 'piecemeal' type (Muller & Kaplan, 1966) of dosage compensation mechanism. There is no reason to assume that, because in mammals the *X*-inactivation is a whole-chromosomal affair (Russell, 1964) with one controlling centre, the *X*-enlargement and *X*-hyperactivity in male *Drosophila* should also be a whole-chromosomal function with one controlling centre. Schultz (1965) and Muller & Kaplan (1966) have found that, unlike mammals, the morphological characteristics of the male *X* in *Drosophila* are not hindered in their expression because of *X*-autosome translocations.

This fact is in agreement with genetic findings that dosage compensators on the *X*-chromosome may not be limited to any particular segment of the same (see Muller, 1950; Stern, 1960). Thus it is possible that individual sets of dosage compensators operating upon individual *X*-linked genes cause them to be hyperactive in the male and that in totality this hyperactivity finds its expression in the increased width and enhanced rate of RNA synthesis by the *X*-chromosome in larval salivary glands of male *Drosophila*.

The evidence obtained in the present work shows very clearly that the hyperactivity of the single male *X* in *Drosophila* is not dependent on the sex-physiology of the individual. If the sex-physiology had a role in causing the normal male *X* to be hyperactive, the single *X* in all *XO* nuclei developing in an *XX* environment should have behaved like the individual asynapsed *X*'s of *XX* nuclei. On the contrary, the single *X* in every *XO* nucleus examined, irrespective of the proportion of such nuclei among total nuclei in the glands, is as much like the two paired *X*'s of the female in width and RNA synthesis as in the regular male (*XY*) and female (*XX*). This cellular autonomy is a clear indication of the fact that the rate of development and other physiological mechanisms have no influence on the enlargement and hyperactivity of the male *X*. The possibility that the rod-*X* is in some way more active than the ring-*X* is not likely, since no significant difference has been found in the widths of the rod- and ring-*X*'s measured separately in each *XX* nucleus. Furthermore, comparison of the number of silver grains scored on single asynapsed rod-*X* in *XX* nuclei with that on the identical region of the rod-*X* in *XO* nuclei shows that the single rod-*X* of *XO* nuclei is nearly twice as active as the single asynapsed rod-*X* of *XX* nuclei.

These observations support the conclusions derived from genetic studies by Sturtevant (1945), Muller (1950), Stern (1960) and others that dosage compensation in *Drosophila* is not mediated by a difference in the physiology of the two sexes. These genetic conclusions were based mainly on studies utilizing the gene *tra* (transformer), which, when homozygous, transforms *XX* females into phenotypic males (Sturtevant, 1945). In such transformed individuals (*X*/*X*; *tra*/*tra*) the expression of all *X*-linked genes examined was identical with that in normal females, not more extreme type, as would have been the case if the male-like physiology has any effect on these dosage relationships. Goldschmidt (1954, 1955) and also Komma (1966) and Lee (1968) have argued that the *X*/*X*;*tra*/*tra* individuals are extreme female-type intersexes and as such physiologically they are more female-like, and that the expression of *X*-linked genes in *X*/*X*;*tra*/*tra* individuals resembles that in normal females is because of female-like developmental physiology and not due to action of the supposed plus and minus compensators. However, the reasons for considering *X*/*X*;*tra*/*tra* individuals to be extreme female-type intersexes may not be fully correct since various early works show that such individuals are, in fact, more male-like from the point of view of fertility (Novitski, 1951), structure of gonads and behaviour during copulation (Seidel, 1963), and sex-comb and other morphological features (Brown & King, 1961; Mukherjee, 1965; R. K. Datta, unpublished). Extensive studies by Brown & King

(1961) and Seidel (1963) clearly establish that these individuals are either completely male-like or intersexes of extreme male-type. These facts suggest that consideration of *X/X;tra/tra* flies as having a female-type sex-physiology is unjustified and therefore the demonstration of sex-independency of dosage compensation mechanism in *Drosophila* by using the gene *tra* cannot be invalidated.

Komma (1966) has presented some evidence to support Goldschmidt's theory of dosage compensation. However, the data are not unambiguous: while the *X*-linked enzyme, glucose-6-phosphate dehydrogenase, behaved in accordance with the expectation, another *X*-linked enzyme, 6-phosphogluconate dehydrogenase did not give the results expected on the sex-dependence theory of dosage compensation. On the other hand, Smith & Lucchesi (1968) have reported the results of a spectrophotometric analysis of *X*-linked eye-colour mutants in *D. melanogaster* in the presence and absence of the gene *dsx* (doublesex), and their results do not support Goldschmidt's idea of sex-dependency of dosage compensation mechanism in *Drosophila*.

In conclusion, it may then be suggested that dosage compensation in *Drosophila* is achieved chiefly, if not fully, by a hyperactivity of the male *X*, in contrast to the *X*-inactivity in female mammals, that this genetic hyperactivity of the male *X* is expressed visibly in the morphology and metabolic activity of the *X*-chromosome in salivary glands of male larvae, and that this hyperactivity and, therefore, dosage compensation in *Drosophila* in general, is not dependent on sex-differentiation, but is a function of the doses of the *X*-chromosome itself.

SUMMARY

Morphology and the rate of RNA synthesis of the *X*-chromosome in *XX/XO* mosaic larval salivary glands of *Drosophila melanogaster* have been examined. For this purpose the unstable ring-*X* was utilized to produce *XX* and *XO* nuclei in the same pair of glands. The width of the *X*-chromosome and the left arm of the 3rd chromosome (3*L*) of larval salivary glands was measured and the rate of RNA synthesis by them was studied upon the use of [^{3}H]uridine autoradiography in such *XX* (female) and *XO* (male) nuclei developing in a female background (i.e. otherwise genotypically *XX*). In such mosaic glands the width of the single *X*-chromosome of male nuclei is nearly as great as that of the paired two *X*'s of female nuclei, as is also the case in normal male (*XY*) and female (*XX*). The single *X* of male nuclei synthesizes RNA at a rate equal to that of the paired two *X*'s of female nuclei and nearly twice that of an unpaired *X* of *XX* nuclei. Neither the developmental physiology of the sex nor the proportion of *XO* nuclei in a pair of mosaic salivary glands of an *XX* larva has any influence on these two characteristics of the male *X*-chromosome.

It is suggested that dosage compensation in *Drosophila* is achieved chiefly, if not fully, by a hyperactivity of the male *X*, in contrast to the single *X* inactivation in female mammals, that this hyperactivity of the male *X* is expressed visibly in the morphology and metabolic activity of the *X*-chromosome in the larval

salivary glands of the male, and that this hyperactivity and therefore dosage compensation in *Drosophila* in general is not dependent on sex-differentiation, but is a function of the doses of the *X*-chromosome itself.

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