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Chemical Induction of Nondisjunction in Drosophila

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Tests for chemically induced nondisjunction and loss of the sex chromosomes in Drosophila were performed. Of 31 compounds tested four gave rise only to an increase of XO exceptions, indicating the induction of chromosome loss. Six compounds, all known spindle inhibitors (colchicine, organic mercury, lead, and tin compounds) gave rise to an increase both of XXY and XO or of only XXY. The effect by metalloorganic compounds of which methylmercury was studied particularly closely, follows a peculiar pattern. In females with structurally normal X chromosomes only an increase of XX gametes is obtained, while with X chromosomes heterozygous for long inversions only O gametes are increased. The data indicates that the effect of the metal compounds occurs at first meiosis and that the process is connected with a meiotic drive, giving rise to a preferential segregation of the two X chromosomes to the functioning pole. The increase only of O gametes with structurally heterozygous X chromosomes can tentatively be explained by a loss due to crossing over within the inversion. An increase of the effect of methyl mercury was obtained where the normal pairing of the X chromosomes was interfered with by means of autosomal inversions. Likewise a synergistic increase of nondisjunction was obtained when a temperature chock of 10°C was applied together with treatment with methylmercury. It is concluded that chemical induction of nondisjunction can be studied in Drosophila, but the sensitivity of the test is rather low and a large amount of material is required.

Introduction

In mutagenicity testing, practical attention has almost exclusively been paid to effects on DNA and the genetic material itself, that is, point mutations and chromosome aberrations. However, in almost all theoretical discussions dealing with the genetic risks of environmental chemicals it is emphasized that one has to take into consideration also chemical effects leading to numerical changes of the chromosomes in the cells. No doubt such a statement is highly justified considering the importance of such chromosomal abnormalities among human congenital diseases. There is a striking discrepancy between theory and practice in this field, however. The reason for this is the difficulties in tackling the problem of numerical alterations of the chromosome number experimentally, which of course is the background of this symposium.

It should be stressed that the origin of a deviating number of chromosomes can vary. There are basically two different mechanisms: one depending on an effect on the chromosomes themselves and one on an effect on extrachromosomal organelles that is the spindle fiber mechanism. The spindle fiber effect implies that a chemical may be totally innocent when it comes to point mutations and chromosomal aberrations, but may affect the chromosomal segregation and therefore still involve a health hazard.

Chemical or physical agents which cause deviating chromosome numbers through direct effects on the chromosomes, do this by chromosomal aberrations, mostly resulting in the loss of chromosomes. Agents which, on the other hand, operate on the spindle fiber mechanism cause both an increase and a decrease of the chromosome number. This distinction is of importance in tracing experimentally the agents affecting the number of chromosomes. The true spindle poisons are of particular interest in the present context, because they are not described in most ordinary mutagenicity tests.

Another matter of importance is the difference in the effect of spindle poisons on meiotic cells as compared to mitotic cells. Although the mechanism of action may be the same in both cases, the effect may differ because of the complicated meitotic process with chromosome pairing, recombination and segre-

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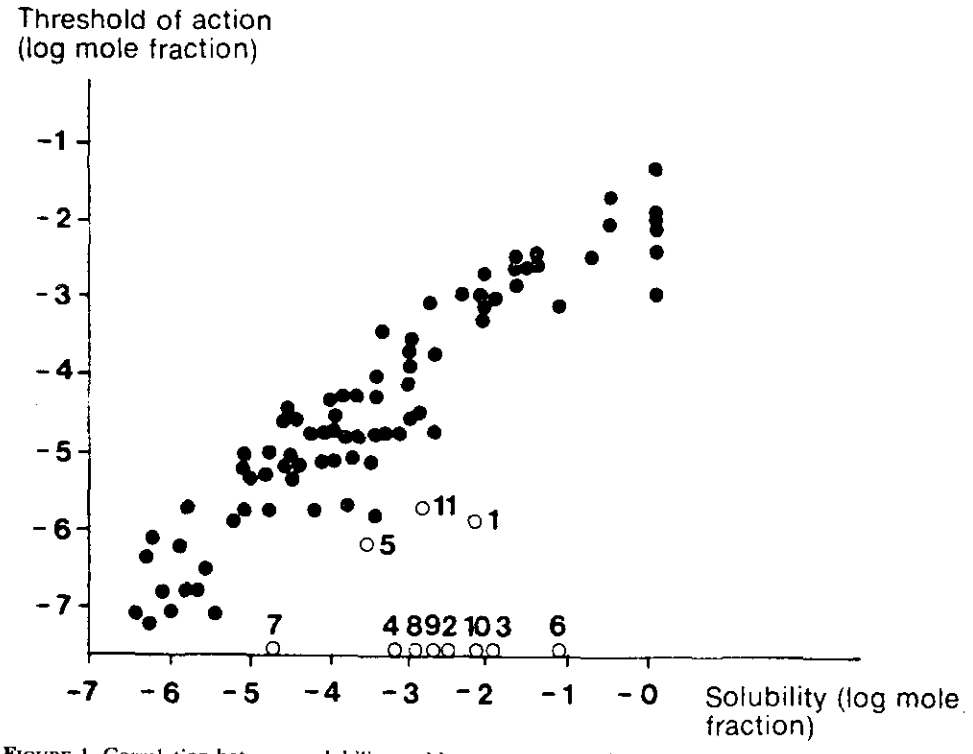

FIGURE 1. Correlation between solubility and lowest concentration for c-mitosis for different organic substances and heavy metals: (1) colchicine; (2) methylmercury dicyandiamide; (3) methylmercury hydroxide; (4) phenylmercury hydroxide; (5) mercury chloride; (6) trimethyltin chloride; (7) tributyltin chloride; (8) diethyllead dichloride; (9) triethyllead chloride; (10) trimethylead chloride; (11) lead nitrate.

gation in meiosis. The action of spindle poisons on meiosis is furthermore of particular interest from a practical point of view, since it can lead to chromosomal diseases of great importance, such as Down's syndrome.

C-Mitosis in Plants

Experimentally, chemical effects on the spindle fiber mechanism are fairly easy to study on mitosis. In fact such studies were performed long before chemical mutagenesis was invented. In the 1940s Levan and Östergren made a series of important studies of colchicine and other chemicals on the spindle fiber mechanism. The standard material they used for their work was Allium root tip cells. Among the results obtained was the finding that a series of organic chemicals cause "colchicine-mitosis" or c-mitosis according to a certain pattern. There was a direct relation between the water solubility of the chemical and the concentration needed to cause c-mitosis (*1, 2*). In other words, almost any chemical should be able to cause c-mitosis provided it is given in sufficient concentration in relation to its water solubility.

There are, however, a number of chemicals which fall outside the regression line for this relation between solubility and "threshold" dose (Fig. 1). Among these there are colchicine and heavy metal compounds (*3, 4*), which have a c-mitotic action at a lower concentration than accounted for by their solubility. It can be assumed that such chemicals act on the spindle fibers by means of a different mechanism than most other chemicals. Presumably chemicals with such a specific c-mitotic action deserve particular attention from the point of view of health hazard.

Dose-Effect Relations

An important matter in connection with these data on c-mitosis in plants, is the dose-effect relation. Toxic action on the spindle fiber mechanism differs from a mutagenic effect on DNA. An established effect on DNA implies an irreversible change of a gene, that is, a unique entity in the cell. Therefore a threshold value similar to the ones encountered for acute toxic effects in general cannot be expected (*5*). An effect on the spindle fiber mechanism, on the other hand, involves identical molecules and or-

ganelles present in large numbers in the cells and therefore a threshold value for c-mitosis and nondisjunction of chromosomes can be expected. The data on c-mitosis in plants by Levan and Östergren (*1*, *2*) also suggests the occurrence of a threshold.

Nondisjunction in Drosophila

Since the discovery by Bridges (*6*) of primary and secondary nondisjunction, Drosophila has constituted an indispensible tool for the study of meiotic nondisjunction. This has been done primarily on the sex chromosomes, but the manipulation of the Drosophila chromosomes during the 1960s made it possible also to study nondisjunction of the large autosomes. This was done especially by the use of attached second and third chromosomes, which caused a permanent maternal or paternal nondisjunction of the large autosomes which could compensate for an induced nondisjunction in the other sex. This test system on the large autosomes has not been used to any extent, however, to study chemically induced nondisjunction, as the method is considerably more difficult than the use of the sex chromosomes for this purpose. The small fourth chromosome can also be used for nondisjunction studies in very much the same way as the sex chromosomes, but has not been done to any appreciable extent for the study of chemical induction of nondisjunction.

Nondisjunction Test of the Sex Chromosomes

Most of the work on nondisjunction in Drosophila thus has been performed by observations of the behavior of the sex chromosomes. By using genetically marked X and Y chromosomes, three effects on the chromosomes can be studied simultaneously in the offspring: nondisjunction, chromosome loss, and induced recombination or translocation involving the Y-chromosome. As is shown in Figure 2, a simultaneous increase of XXY and XO offspring indicates nondisjunction, while an increase of only XO offspring indicates the occurrence of chromosome loss from chromosome breakage. The separation of markers in the two arms of the Y chromosome finally indicates the occurrence of recombination or translocation involving the Y chromosome.

We have used this test system at our laboratory for the screening of chemicals for nondisjunction and chromosome loss. In particular the work has concentrated on the effect of heavy metals.

The following testing procedure has been employed. In most cases the strain used for the work

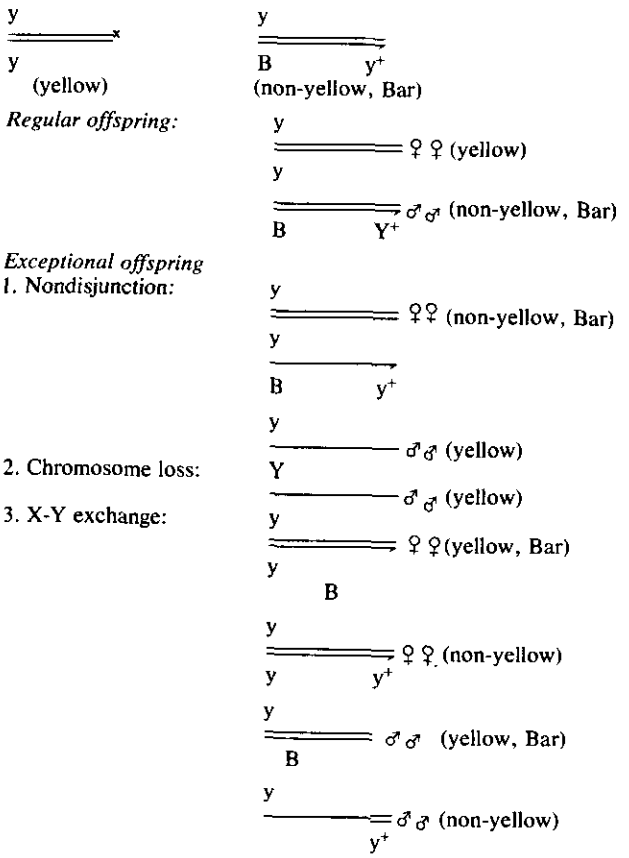

FIGURE 2. Test cross for screening of nondisjunction, chromosome loss, and X-Y exchange.

has been yw^{af}/yw^{af} $xyw^{af}/y^{+}Y$ B^{S}, as shown in Figure 2. The two markers on the Y chromosome are translocated pieces, one carrying the wild type allele of yellow, in one of the arms and one carrying Bar in the other.

The test compound was given to the flies by mixing it into the corn meal-agar substrate. In order to determine an appropriate concentration for the treatment of the flies separate toxicity tests were performed. Different concentrations were then given to larvae, and the time of hatching and survival was measured. A concentration which causes a delay of the hatching, but no increase in the lethality was chosen for the nondisjunction test. With this procedure the delayed hatching indicates that the test substance is taken up by the flies; at the same time, the normal survival ensures that no excessive selection is distorting the results.

In the routine screening for nondisjunction and loss according to the schedule in figure 2, the treatment was given to both sexes during their whole larval period. In most cases the effect in the offspring measured the combined effect in both parents.

Screening Results

The results of the screening of some 30 compounds for nondisjuction and loss are given in Table 1. The separation of the two markers in the Y chromosomes as an indication of recombination or translocation, is also scored as a routine. The frequency, however, is mostly very low and the result rarely contributes any information beyond the data on XXY and XO exceptional offspring. Therefore only the results on nondisjunction and chromosome loss have been included in Table 1. A positive effect for nondisjunction implies a significant increase of XXY offspring, while a positive effect for chromosome loss means an increase only of XO offspring or a larger increase of XO than XXY offspring as compared to the control.

Of the 31 compounds, 11 show association with significant increases of exceptional offspring (Table 1). Of these 11 compounds four only affected the number of XO and may therefore cause chromosome loss without any effect on nondisjunction. It should be mentioned, however, that the increase of XO with Actinomycin D was marginally significant and the result therefore is only indicative.

Seven compounds caused an increase of XXY exceptional offspring, indicating an effect on nondisjunction. The result on quinacrine for XXY is somewhat questionable as the repeats of the experiment did not give a consistent effect on XXY. Therefore it has been put in parentheses. The remaining six compounds are known inhibitors of the spindle fiber mechanism, that is, colchicine and organic heavy metal compounds. The results on heavy metals are of special interest from several points of

Table 1. Results of screening for nondisjunction and loss of sex chromosomes in females and males after treatment of larvae with various compounds in the substrate.

Chemical	Concn in substrate, ppm	Effects on exceptional offspring		No. of flies in test series X 10^3
		XXY	XO	
Actinomycin D	10	–	(+)	14
Adipic acid	4000	–	–	46
Bromobenzene	1000	–	–	26
Cacodylic acid	8	–	–	13
Cadmium chloride	62	–	–	23
Colchicine	2-4	+	+	140
2-Deoxyadenosine	a	–	–	23
2, 4-Dichlorophenoxyacetic acid	100	–	–	72
Diethyllead dichloride	16	+	–	58
1, 2-Dimercaptopropanol	500	–	–	31
Ethylenediaminotetraacetic acid	700	–	+	53
Ethyl methanosulfonate	600	–	–	34
Hexylmercury bromide	4, 6	–	–	109
Lead nitrate	200	–	–	43
Lysergic acid diethylamide	b	–	–	61
Methoxyethylmercury chloride	2, 5	–	–	78
Methylmercury hydroxide	0, 25	+	–	>1000
Methylmetergin	b	–	–	62
Monochlorophenoxyacetic acid	500	–	–	24
Nitrilotriacetic acid	4000	–	+	313
PCB (polychlorinated biphenyls), Clophen A30 + A50	200-250	–	–	152
Pentachlorophenol	400	–	–	73
Phenylmercury hydroxide	0, 25	+	–	49
Polychlorinated 2-phenoxyphenols (predioxins)	1-2	–	+	50
Quinacrine	400	(+)	+	54
Thymidine	c	–	–	25
2, 4, 5-Trichlorophenoxyacetic acid	250	–	–	34
Triethyllead chloride	8	+	–	423
Trimethyltin chloride	0, 12	+	–	27
Tripolyphosphate	4000	–	–	121
Vinyl chloride	d	–	–	36

[a]Adults injected 120 mg/ml.
[b]Adults injected, 1 mg/ml.
[c]Adults injected, 2 mg/ml.
[d]Exposed in air, 200,000 ppm.

view and will therefore be dealt with separately.

The results of this screening of chemicals for nondisjunction and chromosome loss showed that the effect on nondisjunction did not exceed a three- or fourfold increase of the spontaneous frequency. Therefore the test requires that a large number of flies are scored. The narrow range between the concentration required to obtain a significant effect at all and a concentration causing an excessive mortality has been too narrow to permit any successful analysis of the dose effect relation. Therefore only one — as far as possible optimum concentration — of the test substance has been used in most instances.

Effect of Heavy Metals on Nondisjunction

Organic mercury compounds are known as potent c-mitotic agents and inhibitors of the spindle fiber mechanism (*2*). The same is obviously true with alkyl lead (*7*) and other organic heavy metal compounds as well. As is shown in Figure 1, organic compounds of mercury, lead, and tin have a stronger c-mitotic effect than expected from their solubility. Investigations of nondisjunction in *Drosophila* also revealed that organic mercury, lead, and tin increase nondisjunction.

The induction of nondisjunction with heavy metals follows a peculiar pattern. As is apparent from Table 1, the increase of exceptional gametes is restricted to XXY, while no corresponding increase of the reciprocal product of nondisjunction, that is XO, occurs. Particularly because of this unexpected behavior of methylmercury, a detailed analysis of the induction of nondisjunction was performed with this compound (*4, 8*).

Under standard conditions only treatment of females gave any measurable effect by methylmercury. Several tests with females of wild type strains as well as different marker strains have consistently given a similar result (see Table 2) and it must be considered as established that methylmercury only increases XX gametes in females, but not O gametes.

There are experimental results indicating that this effect on nondisjunction can be brought back to a segregation in the first rather than the second meiosis or during premeiotic divisions. No clusters indicating a premeiotic process was observed. The nondisjunction effect wears off according to the excretion of methylmercury, which was studied by means of H^{203}-labeled methylmercury. This finding is consistent with expectation for a meiotic process. An increase of attached X-Y nondisjunction was found after methylmercury treatment, which rules out an effect on second meiosis. No homozygotization was found when using heterozygous markers, which also speaks against an effect on the second meiosis.

A similar pattern of effect was found for trimethyltin. With alkyllead compounds a preliminary study showed a significant increase of XO males after treatment with triethyllead chloride (*7*). However, further experiments on triethyllead and on diethyllead chlorides gave in both cases results consistent with the results on methylmercury that is, there was only an effect on XXY and only a treatment of females was efficient (see Table 3). The reason for this discrepancy between the experiments with triethyllead chloride is not known.

Table 2. Effect of larval treatment with methylmercury hydroxide (0.25 mg Hg/l. substrate) on nondisjunction in females

Exp. no. 2	Parental females	Hg			Control		
		% Exceptions		Total number	% Exceptions		Total number
		XXY	XO		XXY	XO	
1	y w sn/y w sn	0.13[b]	0.18	100734	0.07	0.20	113672
2	y w sn/y w sn	0.07[c]	0.16	76906	0.04	0.16	66863
3	y w sn/y w sn	0.10[c]	0.16	24061	0.05	0.14	35320
4	y w sn/y w sn	0.08[d]	0.15	15446	0.02	0.16	23110
5	y w^a f/y w^a f	0.15[d]	0.19	34150	0.08	0.17	36550
6	y w^a f/y w^a f	0.06	0.02	23250	0.04	0.04	30950
7	Berlin	0.06[d]	0.02	44250	0.02	0.02	75550
8	Berlin	0.05	0.03	44200	0.03	0.04	55800
9	Berlin	0.03	0.02	40100	0.02	0.03	64200
10	Karsnäs 60	0.04	0.02	41100	0.04	0.02	61600
11	Karsnäs 60	0.05	0.04	56150	0.04	0.04	71150

[a]Female crossed to y w sn/sc^8 Y in experiments 1-5; crossed to y w^a f/y^+ Y B^S in experiments 6-11.
[b]$p < 0.001$.
[c]$0.05 > p > 0.01$.
[d]$0.01 > p > 0.001$.

Table 3. Effect of larval treatment with lead compounds on nondisjunction.

Exp. no.[a]	Compound and treatment		Pb % Exceptions XXY ♀♀	Pb % Exceptions XO ♂♂	Pb Number	Control % Exceptions XXY ♀♀	Control % Exceptions XO ♂♂	Control Number
1	$Pb(NO_3)_2$, 200 mg/l.	♀♀ + ♂♂	0.01	0.10	42972	0.03	0.08	54717
2	$(C_2H_5)_2PbCl_2$ 16 mg/l.	♀♀ + ♂♂	0.12	0.09	57527	0.03	0.11	62194
3	$(C_2H_5)_3PbCl$ 8 mg/l.	♀♀ ♂♂	0.11 0.05	0.11 0.10	55890 56122	0.05	0.10	62147

[a]The cross was y w sn/y w sn × y w sn/sn[s] Y in experiment 1 and y w[a] f/yw[a]f × yw[a] f/y[+]YB[s] in experiments 1-6.

Table 4. Effect of larval treatment with methylmercury hydroxide (0.25 mg Hg/l. substrate) on females heterozygous or homozygous for X chromosome inversions.

Exp. no.	Parental females	Hg % Exceptions XXY ♀♀	Hg % Exceptions XO ♂♂	Hg Total number	Control % Exceptions XXY ♀♀	Control % Exceptions XO ♂♂	Control Total number
12	y w sn / In y[4]	0.03[a]	0.42[b]	35933	0.01	0.20	54524
13	y w sn / In y[3P]	0.03	2.17[b]	67858	0.03	1.91	71592
14	In y[4] / In y[3P]	0.04	1.19[b]	52993	0.04	0.99	65056
15	sc cv v car / In sc[S1L] sc[4R]	0.01	2.19[b]	61395	0.02	1.92	40051
16	y w sn / Ins sc[4L] sc[8R]y	0.03	2.88[c]	56903	0.04	2.58	54467
17	y w sn / In EN y	0.02	2.05[c]	52617	0.01	1.83	52618
18	y w sn / In dl 49 y	0.08	0.11	68817	0.07	0.08	56524
19	y ec ct v f / Muller 5 y	0.23[b]	0.45[b]	29053	0.05	0.14	51529
20	In y[4] / In y[4]	0.05	0.30	33803	0.05	0.40[a]	34235
21	In y[3P] / In y[3P]	0.02	0.06	32392	0.03	0.05	35771
22	Muller 5 y / Muller 5 y	0.15[b]	0.05[a]	17576	0.01	0.01	25810

[a]$0.05 \geq p \geq 0.01$.
[b]$p < 0.001$.
[c]$0.01 \geq p \geq 0.001$.

Effect of Methylmercury on Structurally Heterozygous X Chromosomes

Our first work on methylmercury included some experiments with X chromosomes heterozygous for long inversions. It turned out that the effect on such X chromosomes was opposite to that on ordinary X chromosomes as described above. Methylmercury caused only an increase of XO offspring but had no effect at all on XXY offspring (*8*). As shown in Table 4 further experiments with other inversions have verified this finding, and furthermore the increase of XO offspring was consistantly the same and additive

for various long inversions independently of the spontaneous rate of exceptions. The presence or distribution of the proximal heterochromatin does not seem to have any influence on this process. The overall effect by methylmercury was stronger on structurally heterozygous than homozygous chromosomes.

Mechanism of Action of Heavy Metal Compounds

The experimental data on Drosophila indicate that the effect of methylmercury on nondisjunction occurs on first meiosis. From the c-mitotic action of organic mercury and other heavy metals it can furthermore be assumed that this effect on nondisjunction depends on an inactivation of the spindle fiber mechanism that is on the chromosome segregation and not on chromosome pairing. Tests on crossing over in the X chromosomes did not indicate any effect by methylmercury which is in accordance with this assumption.

It may also be pointed out that experimental data show that the recovery of only one type of exceptional offspring does not depend on a viability selection by the methylmercury treatment (*8*).

Although there is no conclusive evidence at hand of the mechanism involved in the differential recovery of nondisjunctional offspring with methylmercury, the results suggest a preferential segregation mechanism. It seems that the nondisjoined X chromosomes after methylmercury treatment are distributed preferentially to that pole during meiosis, which gives rise to the functional gamete and that the effect of methylmercury thus depends on a chemically induced meitotic drive.

The opposite effect, with inversion heterozygotes giving rise only to XO exceptions, may be tentatively explained in the following way. The nondisjoined X chromosomes after the first meiotic division will have a normal cross over frequency within the inversions as the mercury treatment does not alter chromosome pairing and meiotic recombination. Most of the crossovers within the inversions give rise to bridges in second meiosis. Although such bridges might be expected to give rise to nonviable gametes rather than O gametes, according to the results of Novitski (*9*) the situation may be different in the present case, due to a remaining effect of the mercury compound on the spindle fiber apparatus also in second meiosis, which may cause a lagging and elimination of chromosomes involved in bridges.

Experimental Increase of the Sensitivity of the Test System

The effect of methylmercury on X chromosomes heterozygous for inversions differed not only qualitatively from that with homozygous X, but the effect was also higher. It would seem that the structural heterozygocity caused a higher sensitivity to the nondisjunction action of the compound. The effect of methylmercury may therefore be enhanced by a disturbed pairing. This hypothesis was tested by measuring the effect of methylmercury in flies in which the meiotic pairing of the sex chromosomes was affected in some way or another.

In one experiment reported earlier (*8*) on females, X chromosomes heterozygous for Muller 5 inversions were combined with heterozygocity for the Curly inversions in the second chromosome. The interference with meiotic synapsis in two chromosome pairs in this way will enhance nondisjunction in both chromosome pairs, at least partially through a nonhomologous pairing (*10, 11*). It was found that the effect of methylmercury on X chromosome exceptions was enhanced.

The spontaneous nondisjunction of the X- and Y chromosomes in the male is greatly enhanced by the use of X chromosome deficient for the proximal heterochromatin. Treatment with methylmercury of males with such an X chromosome gave a significant increase of exceptional offspring in spite of the fact that no effect can be discerned with standard X chromosomes (*12*).

It has been shown by Tokunaga (*13, 14*) that treatment of females with low temperature (10°C) increases primary nondisjunction of X chromosomes considerably. Experiments were performed in order to find out whether this increase in temperature induced nondisjunction could be used to obtain a higher sensitivity for the nondisjunction test with chemicals. Treatments with 10°C were done according to Tokunaga's procedure. Methylmercury was given to larvae with 0.25 mg/l, substrate. Both structurally homozygous ($y\,f / y\,f \times y\,f / y^{+}\,Y\,B^{S}$) and heterozygous ($y\,w^{a}\,f / y\,Ins\,sc^{4L}\,sc^{8R} \times y\,w^{a}\,f / y^{+}\,Y\,B^{S}$) flies were tested.

The results are summarized in Figure 3. In both types of females there was a considerably stronger effect by methylmercury in combination with low temperature than could be expected from 25°C. The increase was not restricted to one sex; in both types of crosses there is a significant increase of both exceptional XXY and XO offspring. Further analyses are required to get any insight in the possible mechanism for the synergistic effect between temperature and methylmercury treatment.

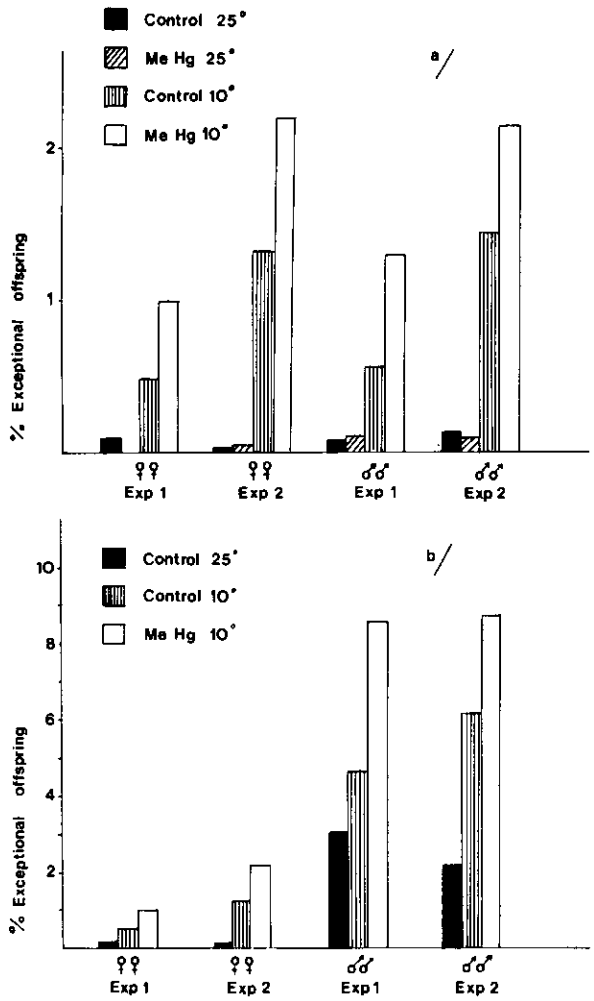

FIGURE 3. Induction of nondisjunction by methylmercury hydroxide in females with and without pretreatment at 10°C for 7 days: (*a*) y w^a f/y w^a f × y w^a f/y^+ Y B^S; (*b*) y w^a f/y Ins sc^{4L} sc^{8R} × y w^a f/y^+ Y B^S; egglaying for 24 hr after temperature treatment.

Conclusions

Nondisjunction tests with sex chromosomes in Drosophila have given positive response to treatments with known inhibitors of the spindle fiber mechanism. The sensitivity of the test system, however, probably is rather low and according to our experience large materials are required in order to discern any effect. A synergistic effect between treatment with organic mercury and genetic or experimental interference with meiotic chromosome pairing indicates the possibility that the sensitivity of the test system may be increased.

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