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Miracil-D: Inhibitor of Ribonucleic acid synthesis and chromosome loss in Drosophila male germ cells.

The effect of Miracil-D (1-diethylaminoethylamino-4-methyl-10-thia-xanthenone), a profound inhibitor of RNA synthesis(1,2), on the production of chromosome loss due to breakage has been investigated. The structural features of this chemical compound are similar (dialkylaminoalkylamino side chain attached to heterocyclic ring system) to those of acridine and actinomycin D which are known to interact with DNA(3,4). The evaluation of genetic damage in this investigation was by the XO method. D. melanogaster males carrying a ring-shaped X-chromosome ($X^{c2}y$ B/Y sc^8 y^+), 4 to 6 hours old, were collected and food withheld for 18 hours. These males were then given Miracil-D (1 mg/ml of regular Drosophila food) for 24 hours prior to mating with 3 day old y w f virgin females. The data on table 1 shows the effect of this chemical treatment. The brood 1 represents those males mated to y w f virgin females for 48 hours continuously. Broods 2 through 7

Table 1. Effect of Miracil-D by feeding and the spontaneous rate of chromosome loss. XO males and mosaics.

Brood		No. of Gametes tested	No. of XO males & mosaics	Percent of XO males & mosaics	Chi-square	Probability
1	Miracil-D	2329	39	1.68		
	Control	2300	26	1.13		
2	Miracil-D	2311	29	1.26		
	Control	2140	30	1.40		
3	Miracil-D	2091	25	1.20		
	Control	2489	27	1.09		
4	Miracil-D	1859	23	1.78		
	Control	2189	21	0.96		
5	Miracil-D	1305	15	1.15	$\chi^2_c = 5.083$	< 0.03
	Control	1193	6	0.50		
6	Miracil-D	1315	23	1.75	$\chi^2 = 2.397$	
	Control	1781	14	0.79		
7	Miracil-D	1480	28	1.89	$\chi^2 = 5.941$	< 0.002
	Control	1498	23	1.54		
Total	Miracil-D	12690	192	1.51	$\chi^2 = 9.588$	< 0.002
	Control	13590	147	1.08		
Total 4 - 7 broods only	Miracil-D	5959	99	1.66	$\chi^2 = 12.107$	< 0.0005
	Control	6661	64	0.96		

Chemical concentration: 1 mg/ml of regular Drosophila food.
Control : regular Drosophila food.

represent consecutive 24 hours re-matings to y w f virgin females. The overall total of these broods (1 through 7) shows about 40 percent increase of XO males and mosaic individuals (due to chromosome breakage and subsequent loss) compared to those in the control group. The data reveals a significant difference between treated and control group (Chi-square of 9.588 with a probability of less than 0.002). In order to calculate those cells affected most prominently, the data on broods 5, 6 and 7 were added. A statistical analysis by 2 x 2 contingency table showed a Chi-square of 12.102 with probability being less than 0.0005. For males

mated daily (or every other day), the first appearance of induced crossing-over which can occur prior to meiosis is in the 7-9 day broods (5). Therefore, the broods 5, 6 and 7 in these experimental series represent those cells affected during the early spermatid and meiotic stages.

The relation of concentration of this chemical in food to incidence of chromosome breaks, in the most sensitive stages, is shown in figure 1. There were three control groups. Control

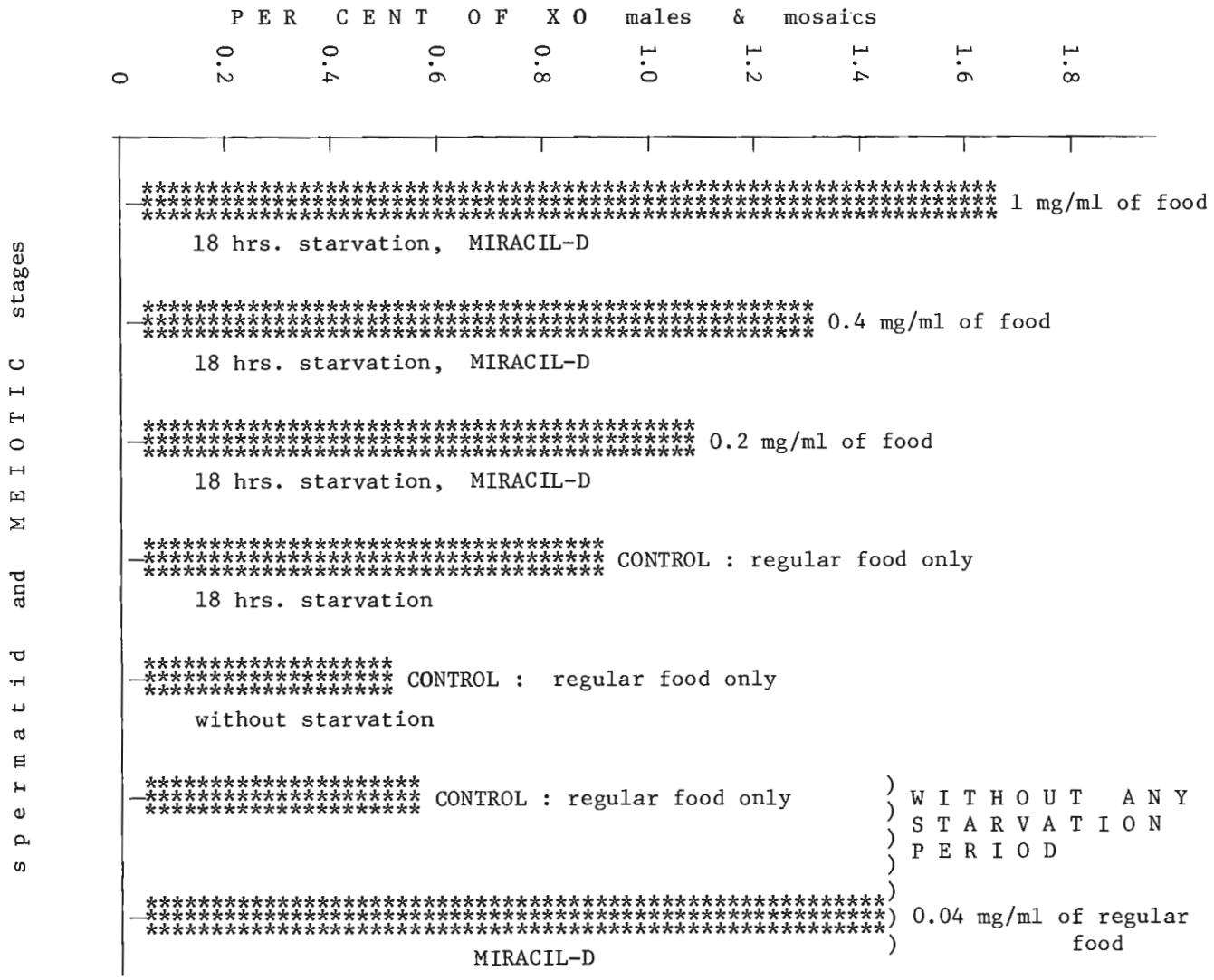

Figure 1. A summary of the various concentration of Miracil-D treatment and the frequency of chromosome loss.

group two had only regular food diet, while the third control group had an 18 hour starvation period prior to returning to the regular food. As seen in figure 1, starvation alone gave some increase in chromosome breaks. Surprisingly, feeding a concentration of 0.04 mg/ml of regular Drosophila food for 24 hours without any starvation period revealed more chromosome breaks than doses five and ten times greater. This may be explained by death of XO males and mosaic individuals from drug toxicity plus starvation in the group receiving higher doses.

These results are similar to those obtained from X-irradiation and from specific inhibitors of DNA synthesis such as mitomycin C(6,7). However, DNA-mediated RNA inhibitor, actinomycin D reduced the frequency of sex-linked recessive lethal mutations in Drosophila(8).

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Gvozdev, V.A., V.J. Birstein and L.Z. Faizullin. Kurchatov Institute of Atomic Energy, Moscow, U.S.S.R. Gene dependent regulation of 6-phosphogluconate dehydrogenase activity of D. melanogaster.

The structural locus Pgd for the 6-phosphogluconate dehydrogenase (PGD) of D. melanogaster has been located on the X-chromosome at 0.64 between the broad (0.6) and prune (0.8).

The variation of Pgd dose from 1 to 2 results in the proportional increase of PGD activity showing the absence of the feed-back regulation. The increase of Pgd dose using $w^{+}Y$ and $Dp(1;3)w^{vco}$ duplications (thrice as much for males and twice as much for females) resulted in 2-3- or 1.5-2.0-fold increase of PGD specific activity in males and females respectively. The PGD activity of normal males and females is twice as much as that of the $Df(1)w^{vco}/+$ and Df(1)Pgd-pn/+ females with a single dose of Pgd.

The quantitative determination of PGD activity in the flies with different doses of Pgd^{A} and Pgd^{A}/Pgd^{B} heterozygotes of either sex show that the gene activity of both alleles in males was twice as much as that of females.

PGD activity in females hyperploid for the distal pieces of X-chromosome (1-3C, 1-9A and 1-9B) including Pgd locus increases for 1.4-1.5 times as compared to that of normal females. Introduction of the 16A1-20 fragment has no effect on PGD activity while 9B-20 and 9E-13C reduces it to 80% level. These results are in accord with Muller's views on the presence of X-linked dosage compensators with negative action.

Chen, P.S. and R. Bühler. Zoologisches Institut der Universität, Zürich, Switzerland. Further studies of the paragonial substance in D. melanogaster.

In our previous study (Chen and Diem. J. Insect Physiol., 7: 289-298, 1961) we located a peptide in the accessory glands (paragonia) of Drosophila male adults. Judging from its mobility on paper chromatogram and amino acid composition it corresponds obviously to the sex peptide found by Fox (Science 129: 1489-1490, 1959). Transplantation of male genital discs into female larvae demonstrated that the synthesis of this peptide is autonomous. This has been confirmed by the recent study of Smith and Bischoff (D.I.S. 44: 122) using the mutant "doublesex". The work done by Garcia-Bellido (Z. Naturf. 19b: 491-495, 1964) showed that grafting of the glands or injection of the paragonial fluid into virgin females resulted in a distinct increase in oviposition. The same results have been reported by Leahy and Lowe (Life Sciences 6: 151-156, 1967). In an attempt to answer the question if the paragonial substance or sex peptide is really the active principle for stimulating egg deposition, methanol extracts were prepared from a large number of male adults and analysed by ion-exchange chromatography. We found that on the amino acid analyzer this peptide was eluted as an acidic component in the region between phosphoserine and glycerophosphoethanolamine. This has been confirmed by fractionation of extracts from a total of 1070 pairs of accessory glands dissected out individually from 8-day-old adult males. On the analyzer the sex peptide appeared as the only prominent peak in the same position revealed by using extracts from whole flies. Injection of the peptide isolated from the column and desalted by high voltage elecrophoresis into virgin females resulted in a two- to threefold increase of oviposition. Our hitherto observation suggested that a single injection is sufficient for the whole adult life. The biosynthesis and turnover of the sex peptide are now under investigation.