Alonso, C. University of Nijmegen, The Netherlands. In situ hybridization of RNA synthesized in larval salivary glands of D. hydei under in vitro conditions.

The hybridization (RNA-DNA) experiments reported here were designed to test a) the capacity for RNA synthesis in isolated salivary glands of Drosophila maintained for three days in a medium described by Poels et al. (1972); b) the possibility that RNA produced by certain puffs is transcribed from repetitive sequences.

Autoradiographs of pulse labeled glands at three days after the onset of incubation revealed a pattern of uridine incorporation into the chromosomes and nucleolus which was essentially the same as seen after in vivo incorporation (Poels et al., 1972). Puff 97A, a puff which becomes active in all cells after explantation of the glands was heavily labeled.

RNA was extracted for hybridization from 70 glands after three days incubation with 50 uCi/150 ul ^{3}H-5T-uridine (spec. act. 27 Ci/mM). The RNA which had a specific activity of 1.1 x 10^{6} dpm/ug, revealed after electrophoresis on SDS-polyacrylamid gels various molecular weight fractions, the major fractions being located at the 28S, 18S and 4-5S positions of the gels. Hybridization experiments were performed with the total RNA extract according to the method of Pardue et al. (1970). Salivary gland squashes were incubated for 16 hrs with the RNA and exposed for 65 days. The autoradiographs consistently revealed labeling of the nucleolus (Fig. 1) and variable labeling of other chromosome areas. Puffed regions, including the puff 97A, did not show consistent labeling.

Fig. 1. Autoradiograph of a salivary gland nucleolus and a part of a chromosome after in situ hybridization of total in vitro synthesized RNA. Exposure time 65 days.

References: Poels, C.L.M., C. Alonso and S.B. de Boer 1972 DIS 48 (this issue); Pardue M.L., S.A. Gerbi, R.A. Eckhardt and J.B. Gall 1970 Chromosoma 29:268-290.

Gavin, J.A. and D.G. Holm. University of British Columbia, Vancouver, Canada. Gamma ray induced nondisjunction of chromosome 2 in females.

Estimating the relative frequency of autosomal nondisjunction (or autosome loss) has been made possible through the construction of compound autosomes (Rasmussen, DIS 34:53). We find, for example, that disomic-2 and nullosomic-2 sperm are regular and frequent products of compound-2 males. Therefore, when these males are crossed with females bearing standard chromosomes, the resultant, but infrequent, progeny are products of exceptional meiotic events during oogenesis. The frequency of nondisjunction increases considerably, however, when crossing-over, in more than one pair of homologous chromosomes, is suppressed by either structural rearrangements or genetic means, or when females are exposed to ionizing radiation. The results of a preliminary study on radiation induced chromosome 2 nondisjunction (and chromosome 2 loss) during oogenesis are recorded in Table 1.

In each of the five tests, the treated (or control) females, whose genotypes are described in Table 1, were divided into groups of 25 and placed in half-pint creamers with 25 C(2L)P, b;C(2R)P, px males. The crosses were carried through five successive broods of three days duration for a total of 15 days. To estimate the overall reproductive potential of the females in each test, a number of bottles involved matings of 25 treated (or untreated) females to males bearing standard second chromosomes. The expected number of progeny listed in Table 1 is an estimate of the total expected progeny if compound males had been replaced by standard males. This value serves as a relative denominator for comparing nondisjunctional frequencies.

Two basically different genetic types of females are being considered: 1) structural

Table 1

Female Parent	Radiation Dose (rads)	Expected No. of progeny	Exceptional Progeny		Nondisjunction	Chromosome loss
			Matro	Patro		
lt stw^3/b pr cn	0	86,000	2	26	4	24
lt stw^3/b pr cn	3500	72,000	17	153	34	136
lt stw^3/SM1	0	84,000	12	26	24	14
lt stw^3/SM1	1000	63,000	208	230	416	22
lt stw^3/SM1	3500	40,000	120	325	240	205

homozygotes for chromosome 2, and 2) structural heterozygotes involving the multiple-break inversion, SM1, Cy. Comparing the results recorded on lines 1 and 3 of the table, we note little difference in the frequency of exceptional progeny from either class of untreated females. The primary contrast is in the ratio of matro/patro progeny. The exceptional progeny produced by structurally homozygous females appear to arise mainly as a function of chromosome loss. In comparing the relative frequencies of exceptional progeny recovered from the different genotypic classes following radiation (from a Co^{60} source at a rate of 65 rads/sec.) we find two quite contrasting results. First, assuming the method of estimating progeny number is reliable, the frequency of induced exceptional meiotic events is considerably greater in structurally heterozygous females than in homozygous females (compare lines 2 and 5 in the Table). The second contrast concerns chromosome loss. Although treated structural homozygotes produce increased numbers of exceptional gametes, we find (as noted on line 2) that most of the progeny are patroclinous. This clearly indicates that chromosome loss is responsible for the majority of the exceptional gametes. The structural heterozygotes, on the other hand, produced more nondisjunctional gametes, although chromosomal loss is significant at 3500 rads. At the lower radiation dose of 1000 rads, all the progeny appear to be products of nondisdunction (i.e., the matro/patro is approximately equal to one). It is also of interest to note that in all but the last experiment (line 5 of the table) the matro/patro ratio remained relatively constant. At 3500 rads (line 5), the matro/patro ratio was quite low in brood 1, indicating high chromosome loss, whereas by brood 5 the ratio increased to approximate unity. We should also note that, since the assortment of the compound-2 chromosomes used in this study approaches randomness in males, the frequency of disomic-2 and nullosomic-2 eggs should be approximately four times the frequency of exceptional progeny.

Mosna, G. and S. Dolfini. University of Milan, Italy. New continuous cell lines of Drosophila melanogaster. Morphological characteristics and karyotypes.

Several continuous cell lines of Drosophila melanogaster have been recently established (Kakpakov et al. 1969; Echalier and Ohanessian 1970; Schneider 1971). We report now on the successful growth of three additional cell lines deriving from embryonic tissues of Drosophila. Two hundred cultures were started from embryos of the wild stock Varese 12-15 hours old, following the technique devised by Echalier and Ohanessian (1970) and using the same D 225 medium. The development of the cultures was similar to that described by Echalier and Ohanessian (1970). The only three cultures, after 8, 10 and 6 months respectively, a new wave of cell multiplication gave rise, by subsequent subcultures, to the three cell lines, called GM_1, GM_2 and GM_3 (Genetics, Milan). Nearly all cells are roundish, only a few are spindle-shaped; the degree of homogeneity in the three lines varies according to the percentage of polyploid cells present in each line.

Karyotypic analysis provided characteristics for distinguishing each line. The preliminary cytological observations are in fact the following:

GM_1 line (16th and 17th passage): A high percentage of cells (75%) are marked by a normal X and a centric heteropycnotic fragment, 10% are XO and the rest are tetraploid. Nearly all cells have only one IV chromosome, the major autosomes being normal (Fig. 1a). Quinacrine staining showed in the short fragment two sections of bright fluorescence, which correspond to the two sections of the normal Y chromosome proximal to the centromere (Zuffardi et al. 1971).