

gonial exchange could have occurred to the left of $su(f)$, since the phenotypes of the exceptional qq were: 2 $y\ ac\ f^+\ mal^+$ and 28 $y\ ac\ f\ mal$. The 1 $y\ ac\ f^+\ mal^+$ q which was bred gave 11 $y\ ac\ v\ mal\ \delta$ offspring and 0 $y\ ac\ f^+\ mal^+$ δ .

Some crossovers in the $su(f)$ to y^+ interval do not involve the bb locus. This is demonstrated by the 6 crossovers in experiment 5 in which the parental qq were heterozygous for the bb deficient $sc^4\ sc^8$ chromosome. At least 1 of these crossovers appears to involve the region between bb and $su(f)$ in the $y^+\ sc^8\ bb\ su(f)\ wa\ sc^8$ chromosome. Whether the heterochromatic region to the left of the bb locus in this chromosome was involved in the other crossovers has not been tested yet.

Possible temperature influence on crossover frequency and changes in redundant sequences. Data presented in the table suggest the possibility that qq cultured at 17 during at least part of their pre-imaginal development may exhibit about twice the amount of crossing over in the $su(f)$ to y^+ interval as qq cultured at the higher temperatures. The 0.116% crossing over in experiment 1a may be an example of this temperature enhancement, since parental qq were inadvertently placed at 17 for 3 or 4 days prior to eclosion. Thus, the maintenance of stock cultures at around 17, in a manner which permits unrestricted crossing over to take place in the heterochromatic region, may serve to promote changes in redundancy not only within the bb locus, but also within repetitive sequences located on either side of the bb locus. The $su(f)$ locus could be included among such structures. The crossover data discussed here has not taken into account the possibility that the wild type and mutant alleles of this locus represent different degrees of redundancy or complexity, and that some of the observed crossovers between $su(f)$ and y^+ may have been exchanges within this locus. These conjectures concerning the organization of $su(f)$ are based upon features previously enumerated (Schalet, Genen en Phaenen, 1970) which include suppression of lz^1 and intensification of lz^{37} by $su(f)^1$, and enhanced by the observations of Voss concerning the l^{3DES} allele of $su(f)$ and $su(l^{3DES})$ reported in DIS 46 and 47.

Comparisons of crossover frequencies in normal and inverted sequences. Crossover frequencies between $su(f)$ and y^+ in normal and inverted sequences under comparable temperature conditions appear to be similar, although the data are too scanty to preclude a 1.5-fold increase. (The 0.4% crossing over at the bb locus in a sc^8 chromosome reported by Schalet (1969) remains an unexplained anomaly.) A perusal of the literature reveals that, almost invariably, crossing over in the segment proximal to $f-B$ in the normal chromosome is increased at least 1.5-fold in the sc^8 (type) chromosome (see also experiment 4 for Tu to $su(f)$ interval), while data based on the segment proximal to car fails to show a consistent comparable increase. Yet, Braver (1956, 1957) reported that the region comparable to the car to y^+ interval in the sc^8 chromosome, namely, the car to w interval in the rst^3 inversion, showed a 3 to 4-fold increase in crossing over as compared to the normal chromosome.

X-ray induced crossing over in inverted sequences. Along with the untreated series of experiments 2, 4 and 5, other qq were exposed to 3,000 R and crossovers, especially between $su(f)$ and y^+ , were scored. For experiments 2 and 4 induced crossovers (2-5.7%) were found for all days of oviposition, except that days 5-6 were not scored. When allowance is made for the difference in X-ray exposure, frequencies and curves were roughly comparable to Roberts' (1969) c(3)G experiment. Induced crossing over did not generate detectable bb locus deficiencies. In experiment 2, when the total exposure was divided into two equal fractions, one hour apart, crossing over was reduced for all days of oviposition and reductions ranged from 14% to 22% below the unfractionated series. In experiment 5, induced crossovers were found only among eggs laid during the first two days of oviposition at a low frequency of about 0.2%.

(This work was carried out within the framework of the Association between Euratom and the University of Leiden, Contract 052-64/2 BIAN, and it also received support from the J.A. Cohen Institute for Radiopathology and Radiation Protection.)

Hanly, E.W. University of Utah, Salt Lake City, Utah. The effect of Actinomycin D on ommatidial bristle development.

In a recent publication (Hanly and Hemmert 1967) the fact that Actinomycin D inhibits the development of *D. melanogaster* ommatidial bristles in vitro was reported. It was noted that when the developing eye (24-30 hr following puparium

formation of Oregon-R at 26°C) attached to the optic lobe of the brain is dissected and cultured in Schneider's medium containing low concentrations of Actinomycin D, ommatidial bristles

gles will not develop, although when placed in medium without Actinomycin, these bristles will grow if done at the proper developmental time. Possible explanations of the very low concentrations needed for inhibition were also presented. Further experiments have given more information on this phenomenon.

Whole brain complexes (cerebral hemispheres, optic lobes and attached eyes) were dissected 25 1/2 hr after puparium formation and placed in approximately 1 ml Schneider's medium containing Actinomycin D (Merck, Sharp & Dohme) to 0.075 μ g/ml. They were removed with a fine pipette after 5 min and washed twice in 2 ml each of Schneider's medium without the antibiotic. They were then placed in hanging drop cultures containing no inhibitor as described in Hanly, Fuller and Stanley (1967) and observed for a 72 hr period. Appropriate controls were also cultured.

Eyes with attached optic lobes, but without cerebral hemispheres, competent to form ommatidial bristles (27 1/2 hr after puparium formation) were also dissected and treated in like manner. Eyes alone were similarly tested by dissecting at 28 1/2 hr after puparium formation. Controls in these cases consisted of one side of a complex while experimentals were the opposite side.

In all cases examined, ommatidial bristles developed in the controls while none appeared in the treated eyes (approximately 100 cultures). Other developmental processes occurring in the eye and brain during this time (growth of the ommatidia, apparent deposition of pigment granules, extension of the outer optic glomerulus, and growth of the optic nerve) did not seem to be affected by the antibiotic treatment. Subsequent deposition of normal in vitro amounts of corneal material was also apparently not affected. These results indicate that RNA synthesis necessary for developmental functions other than ommatidial bristle synthesis has occurred prior to or occurs after the pulses of Actinomycin D; or that the DNA locus or loci responsible for bristle synthesis are particularly sensitive to low concentrations of the inhibitor.

Cultures were also established in order to determine the sensitivity of optic lobes with attached eyes over different time periods of development. These tissues were dissected, with appropriate controls, every half hour from 27 1/2 through 33 hr. Eye-optic lobe complexes dissected at 29 1/2 hr following puparium formation and treated with Actinomycin D developed very short, fine bristles. Those dissected and treated at 30 hr had slightly longer and larger bristles. Each subsequent half hour produced longer bristles until normal-sized bristles were produced in cultures of eyes dissected at 31 hr. Bristles normally appear in vivo between 32 and 33 hr at 26°C following puparium formation. These results indicate that at least some DNA-dependent RNA necessary for ommatidial bristle development is synthesized approximately 2 1/2 - 3 hr before its apparent function is seen. Fristrom (personal communication) and others have calculated a "half-life" of some messenger-type RNA molecules in *Drosophila* to be between 2 1/2 and 3 1/2 hr. Furthermore, it appears that for normal bristle development, continued RNA synthesis is necessary for approximately 1 1/2 hr. This could be a developmental "maintenance" process.

During the course of these experiments it was noted that if concentrations of Actinomycin D of 0.05 μ g/ml were used, or if the exposure period used was less than 5 min, some ommatidial bristles would develop. They were, however, usually abnormal: irregular, short and stubby, multiple bristles from single sockets, or bent and crooked. Many of these appeared similar to those in the singed phenotype.

References: Hanly, E.W., C.W. Fuller and M.S. Stanley 1967, The morphology and development of *Drosophila* eye. I. In vivo and in vitro pigment deposition. *J. Embryol. exp. Morphol.* 17:491-499; Hanly, E.W. and W.H. Hemmert 1967, Morphology and development of the *Drosophila* eye. II. In vitro development of ommatidial bristles. *J. Embryol. exp. Morphol.* 17:501-511.

This work was supported by grant GM-16646 from the Public Health Service.

Mitra, N. and A.S. Mukherjee. University of Calcutta, India. Continuous ^3H -TdR labeling pattern as the beginning of replication cycle: further evidence.

The results of the present experiment throw some light on the controversy regarding the type of labeling pattern at the initiation of the DNA replication cycle in the polytene chromosomes of *Drosophila melanogaster*. Ninety-hour old, late third instar larvae were fed for 2 hr on mitomycin C mixed in the medium at a conc. of 0.02 ml/gm of food (conc. 2 mg/ml). Excised salivary glands were subjected to autoradiography after a 20 min incubation in 200 $\mu\text{Ci/ml}$ of ^3H -

mycin C mixed in the medium at a conc. of 0.02 ml/gm of food (conc. 2 mg/ml). Excised salivary glands were subjected to autoradiography after a 20 min incubation in 200 $\mu\text{Ci/ml}$ of ^3H -