THE DISTRIBUTIVE EFFECT OF SELENOAMINOACID TREATMENT ON CROSSING-OVER IN *DROSOPHILA MELANOGASTER*

KEH P. TING¹ AND GEORGE W. R. WALKER

Department of Genetics, University of Alberta, Edmonton, Alberta, Canada

Received January 9, 1968

SELENIUM is known to be readily incorporated in place of sulfur into the proteins of many species of organisms (see JÁUREGUI-ADELL 1966, for review); the selenohydryl group (of selenocysteine) is less reactive than the corresponding sulfhydryl (PAINTER 1941; NICKERSON, TABER and FALCONE 1956). The authors recently found that treatment with sodium selenate before meiosis decreased the rate of recombination in barley and caused structural alterations in the meiotic chromatin (WALKER and TING 1967). They tentatively concluded that these effects were probably mediated by alterations in the physicochemical properties of cellular proteins in which selenium was incorporated. The suggestion was made that selenium could reduce strand breakage either by relaxing the chromosomal protein, with a consequent lessening of physical stress in the chromosome fibrils, or by altering the structure of enzymes effecting strand breakage. Since these alternatives might be tested by determining whether or not changes in the distribution of crossovers occurred with treatment, a four-point linkage study in *Drosophila melanogaster* was undertaken. The two selenoaminoacids selenocysteine and selenomethionine were chosen to replace the sodium selenate used in the previous study.

MATERIALS AND METHODS

Crossing-over was measured in the *X*-chromosome with the aid of the markers γ (0.0), *cv* (13.7), *v* (33.0) and *f* (56.7) described in BRIDGES and BREHME (1944). The region covered includes most of the euchromatin, but does not extend into the heterochromatin (see Figure 5). Two high-viability aberration-free stocks were used, one a multiple-marker stock from the Department of Genetics, University of Wisconsin, the other a wild-type Oregon-R stock from the Department of Biology, Yale University. A standard testcrossing procedure was carried out in quarter-pint bottles at a constant temperature of $25 \pm 1^\circ\text{C}$. Virgin γ *cv* *v* *f*/ γ *cv* *v* *f* females were mated with ++++/Y males on selenocysteine or selenomethionine-enriched and on unenriched media, to obtain treatment and control tetrahybrids. The culture media were prepared as follows: Selenocysteine concentrations of 1.63, 5.22, 16.3, 81.6 and 327 μM and selenomethionine at 1.63, 5.22 and 16.3 μM were prepared in acetic acetate buffer, pH 4.7. These treatment solutions, and a control consisting of the buffer alone, were used in place of distilled water in the recipe (water, molasses, corn meal, brewer's yeast, agar and moldex) of the Biology Division, Oak Ridge National Laboratories, U. S. A.

Experiments I and Ia designate dual replicates of the selenocysteine series of six cultures (including control), performed in 1966 and 1967, respectively. It should be noted that although all controllable factors were faithfully reproduced, nutrient batches were different, and atmospheric

¹ Present address: Division of Biology, California Institute of Technology, Pasadena, California 91109.

humidity and gas content were not under control. Experiment II designates a single run of the selenomethionine series of three cultures, carried out at the same time as experiment I, with the same solid-nutrient batch, and in the same incubator; unfortunately, a separate control for this experiment was not provided for.

Virgin tetrahybrid females were isolated, within 10 hrs of first emergence, from each of the cultures, in sufficient numbers to allow several group-matings to be made, at the end of a 24 hr period following first emergence. Each group-mating consisted of four tetrahybrid females and four tester males, on selenoaminoacid-free medium in a standard bottle. The flies were transferred to fresh bottles at the end of a 3-day period, and this process was continued at 3-day intervals until 4 broods of testcross progeny had been established from each mating group. These progeny were then scored for phenotype and sex, and the data were subjected to a differential viability test in which the frequencies of the two reciprocal types of recombinants for each type of segregational event were compared for parity (see RESULTS). Since males in experiments I and II were found to be more sensitive than females to viability effects, the scoring data for males were not used for further analysis, and in experiment Ia females were excluded for the same reason.

Linkage estimates (*viz.*, recombination frequencies and map distances) and their standard errors were calculated from the selected scoring data by the Maximum Likelihood method (cf. BAILEY 1961) and were subjected to variance and regression analyses (cf. MATHER 1965) for initial assessment of treatment effects on these parameters. In addition, the frequencies of single- and double-crossover progeny were subjected to separate, more detailed analyses of variations in the intensity distribution of crossing-over along the γ - f region. For these analyses, a third set of parameters, the ratios of the double-crossover chromosome frequencies for different segment-pairs, was derived from the double-crossover frequencies.

Single- and double-crossover chromosome frequencies, symbolized as SE and DE (E = exchange) were estimated from scoring-data (see Table 2) as follows:

(1) for any segment i ($= 1, 2$ or 3), $SE_i = a_i/n$, where a_i is the number of individuals showing a single crossover only, located in segment i , and n is the total number of the testcross progeny;

(2) for any pair of segments, i, j ($= 1, 2; 1, 3; \text{ or } 2, 3$), $DE_{i,j} = a_{i,j}/n$, where $a_{i,j}$ is the number of individuals showing a crossover in each of the segments i and j , but none in the remaining segment.

(3) for the total assessed region, consisting of segments 1, 2 and 3, $SE_{s.c.} = SE_1 + SE_2 + SE_3$, and $DE_{s.c.} = DE_{1,2} + DE_{1,3} + DE_{2,3}$.

Relative frequencies, relating one type of double-exchange event with another, and symbolized as RDE, were calculated from the DE estimates as follows:

for any segment triple i, j, k , $RDE_{i,j/i,k} = DE_{i,j}/DE_{i,k}$.

OBSERVATIONS AND RESULTS

Physiological effects of treatment: Treatment of eggs and larvae at concentrations of either selenoaminoacid up to and including $16.3 \mu\text{M}$ resulted in no detectable larval or pupal mortality, and those parents feeding on selenoaminoacid-enriched media showed no falloff in yield. At $81.6 \mu\text{M}$ selenocystine, however, a slight reduction in adult yield was found, and at $327 \mu\text{M}$ in experiment I, larval and pupal lethality were very high; from six mating bottles prepared, only 20 larvae survived to pupate, and only 4 flies (3 males and 1 female) emerged. Moreover, oviposition to emergence occupied twice the normal period, and all the adults, which gave barren matings, were half the size of normal adults. Similar effects, noted with zinc deficiency (STEFFENSEN 1957) indicate an effect on metabolically active proteins (see DISCUSSION).

Differential viability in testcross offspring: A compilation of the selected scoring

TABLE 1
Differences in viability between reciprocal exchange classes in experiment I females
and Ia males testcross progeny

	γ	cv	v	f	Experi- ment I (females)	Experi- ment Ia (males)	Differential ratio of pooled data		γ	cv	v	f	Experi- ment I (females)	Experi- ment Ia (males)	Differential ratio of pooled data
no exchange	γ	$+$	$+$	$+$	2198	2498	.85 : 1*		γ	$+$	v	f	68	31	1.11 : 1
	$+$	$+$	$+$	$+$	2680	2829			$+$	cv	$+$	$+$	59	30	
	γ	$+$	$+$	$+$	726	677		two exchanges	γ	$+$	$+$	f	215	199	.96 : 1
	$+$	cv	v	f	608	539	1.22 : 1*		$+$	cv	v	$+$	218	213	
one exchange	γ	cv	$+$	$+$	1059	1025	.95 : 1		γ	cv	$+$	f	179	215	.90 : 1
	$+$	$+$	v	f	1096	1090			$+$	$+$	v	$+$	204	234	
	γ	cv	v	$+$	830	840		three exchanges	γ	$+$	$+$	$+$	12	3	1.25 : 1
	$+$	$+$	$+$	f	834	896	.97 : 1		$+$	cv	$+$	f	7	5	

* Difference from a 1 : 1 ratio highly significant.

data for experiment I females and Ia males, showing the differentials in viability between the reciprocal classes for each type of exchange event, is presented in Table 1.

Highly significant differences from a 1:1 ratio for the first two reciprocal-class pairs are traceable largely to a detrimental effect of *cv*, which, except in the second 2-exchange group, is always present in the less-frequent class. Unfortunately, no valid adjustment for this distortion is possible. The effects of the remaining mutant alleles may be considered negligible (for example, when the unweighted reciprocal-class ratios of mutant : wild type for each segregating allele are averaged, the fractions .99 for $\gamma/+$, .99 for $\nu/+$, and .96 for $f/+$, are obtained, in contrast with .92 for *cv/+*).

A more detailed study of the reciprocal-class ratios for each of the five treatments revealed no consistent trends with increasing dosage, thus there are no evidences of any genotype-specific residual treatment effects on viability that might distort linkage estimates.

Effects of treatment on linkage estimates: The selected scoring data, with reciprocal-exchange classes combined, are shown in Table 2, and the linkage estimates derived from these scores are given in Table 3. The percentage standard error, for the individual concentration-brood values, averages 8% for recombination percentages, and 3% for the γ -*f* map-distances.

Variance analyses, performed on single-brood estimates from Table 3 for the partition of variance into treatment and brood components, are summarized in Table 4. The analysis of map-distance for the γ -*f* region indicates a significant treatment effect in experiment I, and a like effect in experiment II, but no significance for experiment Ia. Supporting evidence for the validity of effects in I and II is that there is a reasonably close linear correlation of the twelve sets of paired concentration-brood values ($r = .523$) between the two experiments. This is reflected in their regression lines Y_I and Y_{II} , derived from Table 3 data with controls (carrying unknown amounts of trace selenium) excluded, and illustrated in Figure 1. Since both cystine and methionine may readily be converted to cysteine in some organisms, such a relatedness of effect might be expected in these experiments. The authors therefore conclude that a real treatment effect exists. Although the non-significance of treatment variance in experiment Ia

TABLE 2
Selected scoring data for experiments I, Ia and II

Treatment and sex of progeny	Concentration (μM)	Brood	Numbers of testcross progeny								Total progeny (n)
			Non-crossover	Crossover							
				Segments showing crossingover							
				1	2	3	1,2	1,3	2,3	1,2,3	
Experiment I: selenocystine (females)	Control	1	235	52	99	85	6	20	24	0	521
		2	250	60	120	81	7	20	20	0	558
		3	247	81	121	100	5	29	27	2	612
		4	210	66	98	77	5	14	12	1	483
	1.63	1	226	66	89	88	10	25	26	1	531
		2	284	92	116	97	2	30	26	2	649
		3	126	52	61	40	2	12	12	0	305
		4	180	57	88	79	6	24	20	2	456

TABLE 2—Continued

Selected scoring data for experiments I, Ia and II

Treatment and sex of progeny	Concentration (μ M)	Brood	Non-crossover	Numbers of testcross progeny							Total progeny (n)
				Crossover							
				Segments showing crossingover							
				1	2	3	1,2	1,3	2,3	1,2,3	
Experiment Ia: selenocystine (males)	5.22	1	514	134	209	158	11	47	39	2	1114
		2	327	91	171	110	11	25	22	1	758
		3	136	49	59	55	6	13	15	1	334
		4	343	97	165	101	18	37	25	2	788
	16.3	1	229	57	108	90	2	11	11	1	509
		2	218	54	84	70	9	17	21	3	476
		3	108	38	47	27	6	12	5	0	243
		4	162	54	70	51	7	23	13	0	380
	81.6	1	281	50	105	97	2	21	11	0	567
		2	331	69	130	93	7	22	24	1	677
		3	255	58	118	81	3	12	19	0	546
		4	216	57	97	84	2	19	11	0	486
	Control	1	260	53	112	102	5	19	21	0	572
		2	240	52	111	71	3	23	34	0	534
		3	288	75	122	76	5	28	32	1	627
		4	259	61	110	81	1	19	21	0	552
	1.63	1	290	57	105	115	6	15	28	1	617
		2	253	72	108	74	0	16	21	3	547
		3	237	69	96	66	4	27	17	0	516
		4	262	51	123	87	0	20	20	0	563
	5.22	1	286	39	98	99	1	25	29	2	579
		2	301	51	112	78	4	27	12	0	585
		3	269	58	79	91	4	18	24	0	543
		4	229	69	80	81	1	22	21	0	503
	16.3	1	308	73	121	93	5	21	25	0	646
		2	257	54	111	87	3	24	22	0	558
		3	262	72	92	92	1	15	17	0	551
		4	247	55	116	81	2	18	13	0	532
	81.6	1	263	57	105	94	7	14	31	1	572
		2	219	75	106	84	5	16	26	0	531
		3	309	53	105	102	1	21	17	0	608
		4	288	70	103	82	3	24	18	0	588
	Control										
Experiment II: selenomethionine (females)	1.63	1	118	38	64	48	3	14	12	0	297
		2	116	40	55	43	1	17	9	0	281
		3	75	24	39	23	2	12	5	0	180
		4	65	26	21	25	5	10	7	1	160
	5.22	1	303	83	139	99	10	31	23	0	688
		2	200	68	99	67	3	16	24	0	477
		3	214	73	91	73	6	20	13	1	491
		4	212	73	108	72	9	25	26	0	525
	16.3	1	301	78	148	121	8	26	31	1	714
		2	197	73	83	85	8	20	14	0	480
		3	185	59	85	77	7	19	12	1	445
		4	174	52	76	62	9	13	15	1	402

TABLE 3

Linkage estimates from experiments I, Ia and II

Region	Concentration (μ M)	Experiment I					Experiment Ia					Experiment II				
		Broods				All broods	Broods				All broods	Broods				All broods
		1	2	3	4		1	2	3	4		1	2	3	4	
Recombination percentages																
γ - <i>cv</i>	Control	15.0	15.6	19.1	17.8	16.9	13.5	14.6	17.4	14.7	15.1	...	...	...	...	...
	1.63	19.2	19.4	21.6	19.5	19.7	12.8	16.6	19.4	12.6	15.2	18.5	20.6	21.1	26.3	21.0
	5.22	17.4	16.9	20.7	19.5	18.2	11.6	14.0	14.7	18.3	14.5	18.0	18.2	20.4	20.4	19.2
	16.3	14.0	17.4	23.0	22.1	18.3	15.3	14.5	15.9	14.1	15.0	15.8	21.0	19.3	18.7	17.2
<i>cv</i> - <i>v</i>	81.6	12.9	14.6	13.4	16.0	14.2	13.8	18.1	12.3	16.5	15.1	...	...	...	...	...
	Control	24.8	26.3	25.3	24.0	25.2	24.1	27.7	25.5	23.9	25.3	...	...	...	...	...
	1.63	23.7	22.5	24.6	25.4	23.9	22.7	24.1	22.7	25.4	23.7	26.6	23.1	25.6	21.3	24.4
	5.22	23.4	27.0	24.3	26.6	25.3	22.5	21.9	19.7	20.3	21.1	25.0	26.4	22.6	27.2	25.4
<i>v</i> - <i>f</i>	16.3	24.0	24.6	23.9	23.7	24.1	23.4	24.4	20.0	24.6	23.1	26.3	21.7	23.6	25.1	24.4
	81.6	20.8	23.9	25.6	22.6	23.3	25.2	25.8	20.2	21.1	22.9	...	...	...	...	...
	Control	24.8	21.7	25.8	21.5	23.6	24.8	24.0	21.9	21.9	23.1	...	...	...	...	...
	1.63	26.4	23.9	21.0	27.4	24.9	25.8	20.8	21.3	22.6	22.7	24.9	24.6	22.2	26.9	24.6
γ - <i>f</i>	5.22	22.1	20.8	25.0	20.9	22.0	26.8	20.0	24.5	24.7	23.9	22.2	22.4	21.8	23.4	22.5
	16.3	22.2	23.3	18.1	22.9	22.1	21.5	23.8	22.5	21.1	22.2	25.1	24.8	24.5	22.6	24.4
	81.6	22.8	20.7	20.5	23.5	21.7	24.5	25.7	23.0	21.1	23.1	...	...	...	...	...
	Map distances															
γ - <i>f</i>	Control	64.5	63.6	70.1	63.4	65.6	62.4	66.3	64.8	60.5	63.5	...	...	...	...	...
	1.63	69.3	65.8	67.2	72.4	68.5	61.3	61.6	63.4	60.6	61.7	70.0	68.3	68.9	74.4	70.0
	5.22	62.9	64.8	70.1	67.1	65.3	60.1	55.9	58.9	63.2	59.6	65.3	67.1	64.8	71.0	66.9
	16.3	60.1	65.3	65.0	68.7	64.4	60.2	62.7	58.4	59.8	60.3	67.2	67.7	67.4	66.4	67.2
	81.6	56.4	59.2	59.5	62.1	59.2	63.5	67.6	55.6	58.7	61.1	...	...	...	...	...

(Table 4) and a horizontal regression line (Figure 1) may cast doubt on this assessment of experiments I and II, it should be pointed out that even the effects seen in these experiments are slight. Indeed, in experiment Ia the regularity of

TABLE 4

Summary of analyses of variance and of regression for map distance

		Selenocystine									
Region	Item	N	MS	Experiment I			N	MS	Experiment Ia		
				t	P	b			t	P	b
<i>γ-f</i>	Brood	3	20.05	1.76	NS*	...	3	6.77	.81	NS	...
	Concentration	4	47.63	2.72	<.02	—2.68	4	9.22	.94	NS	.006
	Error	12	6.46	...	...	...	12	10.37	..	...	...
		Selenomethionine									
Region	Item	N	MS	Experiment II							
				t	P	b					
<i>γ-f</i>	Brood	3	63.04	13.2	<.001	...					
	Concentration	2	21.40	4.5	<.001	—1.60					
	Error	9	4.78	..	...	...					

* No significance.

FIGURE 1.—Regression of map distance on selenoamino acid concentration (logarithmic scale).

the trends in the 4-brood averages, and the close correlation of the values for the early-brood trends (the eggs producing later broods had probably undergone meiosis during a normal nutrition of adult females) provide evidence for a treatment effect here as well. For this reason, therefore, all three experiments are considered in the further analysis of treatment effects.

Effects of treatment on exchange pattern: Table 5, computed directly from the scoring data of Table 2 after a pooling of the four broods from each treatment, shows the frequencies of the different types of exchange chromosomes, together

TABLE 5

Segmental frequencies of single- and double-exchange and double-exchange ratios, from pooled 4-brood data

Experiment	Concentration (μM)	Doubles				Ratios of doubles*			Singles		
		1,2	1,3	2,3	All seg'ts	1,2/1,3	1,2/2,3	2,3/1,3	1	2	3
I	Control	.011	.038	.038	.087	0.28	0.28	1.00	0.119	0.201	0.158
	1.63	.010	.047	.043	.100	.022	0.24	0.92	0.138	0.182	0.157
	5.22	.015	.041	.034	.090	0.37	0.46	0.83	0.124	0.202	0.142
	16.3	.015	.039	.031	.085	0.38	0.48	0.79	0.126	0.192	0.148
	81.6	.006	.033	.029	.067	0.20	0.21	0.88	0.103	0.198	0.156
Ia	Control	.006	.039	.047	.092	0.16	0.13	1.21	0.105	0.199	0.144
	1.63	.004	.035	.038	.078	0.13	0.12	1.10	0.111	0.193	0.152
	5.22	.005	.042	.039	.085	0.11	0.12	0.93	0.098	0.167	0.158
	16.3	.005	.034	.034	.073	0.14	0.14	0.98	0.111	0.192	0.154
	81.6	.007	.033	.040	.080	0.21	0.17	1.22	0.111	0.182	0.157
III	Control	.011	.038	.038	.087	0.28	0.28	1.00	0.119	0.201	0.158
	1.63	.010	.047	.043	.100	.022	0.24	0.92	0.138	0.182	0.157
	5.22	.015	.041	.034	.090	0.37	0.46	0.83	0.124	0.202	0.142

* These ratios were calculated without rounding-off the fractions for doubles.

with the RDE ratios. Standard errors $\gamma(1-\gamma)/n$ for the SE and DE estimates (γ) range approximately in magnitude from .2 to .05 of the estimates themselves, the maximum errors (except for 1.63 μM , experiment II) being, for SE_1 's, .003; for SE_2 's, .005; for SE_3 's, .005; for $\text{DE}_{1,2}$'s, .007; for $\text{DE}_{1,3}$'s, .008; for $\text{DE}_{2,3}$'s, .010; and for DE_T 's, .019, respectively. Average frequencies over all treatments for any experiment carry errors approximating half the above values. Thus, a comparison of ranges of variation for each frequency with treatment with these errors reveals that the ranges are, in general, considerably greater than would be expected of random variation.

FIGURE 2.—Frequencies of single-exchange chromosomes for segments 1, 2 and 3, respectively, following treatment with various concentrations of selenoamino acids (experiment I and Ia, selenocystine; experiment II, selenomethionine).

The SE curves are presented in Figure 2. An interesting feature is a reversal of inflection, seen at each experimental point, when curves for different segments are compared (for example, when the SE_1 and SE_2 curves for experiment I are compared, peaks are seen in SE_1 at concentrations for which troughs occur in SE_2 , and vice versa). Except for $1.63 \mu M$, experiment Ia, for any experiment two SE curves are always concordant, and discordant with the third, for all points. This suggests differential effects, at each concentration level, in different regions of the chromosome. Similar concordances (in SE_1) and discordances are seen between the experimental group (I, II) and experiment Ia. It is interesting to note, however, that the total frequency of single exchange progeny remains quite stable over the treatment range. The maximum and minimum frequencies (showing descending order in experiment I only) are for experiment I, .478–.456; for Ia, .457–.423; and for II, .489–.479). It would appear, in fact, true that the frequencies of non-crossover chromosomes, as well, are little changed by treatment, and that the more noticeable overall effects lie rather at the other end of the exchange-pattern spectrum (*viz.* in double-crossover events). Such effects are seen in the DE curves, shown in Figure 3, in which a clearly defined trend is to be seen for the total segment: a general decline in DE_T with treatment concentration. An initial increase from control to $1.6 \mu M$ selenoaminoacid in experiment I (and in experiment II, if the control value of experiment I is considered as a control for this experiment too) appears to be mainly due to an increase in $DE_{1,3}$, and the subsequent decline to reduction in $DE_{2,3}$ and $DE_{1,3}$, with a delayed reduction in $DE_{1,2}$.

Since this initial increase is totally absent in the curves of experiment Ia, and since the authors were unable to obtain estimates of the amount of selenium present in the control media, it was decided that the range of effects below $1.63 \mu M$ should be ignored for the remainder of this analysis.

On the assumption that the DE_T curve of experiment I, like the map-distance curve, is essentially linear in the dosage region from $1.63 \mu M$ to $81.6 \mu M$, it may be seen that the $DE_{1,2}$ curve constitutes a noticeably convex component, while the $DE_{2,3}$ is noticeably concave and the $DE_{1,3}$ linear. This suggests an enhancement of $DE_{1,2}$ at the expense of $DE_{2,3}$. The high error for $1.63 \mu M$ in experiment II and obvious fluctuations in DE_T for Ia preclude an evaluation of intersegmental effects from the DE curves for these experiments, and resort to another means of evaluation is required.

This is provided by the RDE curves, shown in Figure 4. These trace the relative tendencies of two segments for coincident exchange with the remaining segment (appearing as subscript in both numerator and denominator of the RDE fraction), and thus may be regarded as describing relative interference. Except for the experiment II curve (suspected of error at $1.63 \mu M$) they show a marked and uniform curvature, indicative of continuous and directed changes in the interference pattern of the chromosome with dose (for effects in the control— $1.63 \mu M$ region in the first two curves of experiment I see DISCUSSION) and are therefore suited to logical analysis.

Thus, if the slope from $1.63 \mu M$ to $5.2 \mu M$ is taken as the initial slope of these

FIGURE 3.—Frequencies of double-exchange chromosomes for the γ - f region and for the segment-pairs 1 and 2, 1 and 3, 2 and 3, following treatment with various concentrations of selenoamino acids (experiment I and Ia, selenocystine; experiment II, selenomethionine).

curves (that portion from control to 1.63 μM being ignored) it is seen from the three curves for experiment I that the tendencies of segment 3 for coincident exchange decreases in relation to those of 1 and 2, and that of segment 2 decreases with relation to 1. Hence the relative decline is greatest for 3 and least for 1. Calculations using the Table 5 values show that the frequency of such coincident crossovers falls in this order (.090 to .075 for 3; .053 to .049 for 2; .057 to .056 for 1). A parallel analysis of experiment Ia indicates that there is a relative enhancement of coincident crossovers in segments 1 and 3 when compared with 2,

FIGURE 4.—Ratios of double-exchange frequencies between segment pairs following treatment with various concentrations of selenoamino acids (experiment I and Ia, selenocystine; experiment II, selenomethionine).

also confirmed by calculations (.039 to .047 for 1; .073 to .081 for 3; .042 to .044 for 2).

The overall curvature of these two sets of curves suggests a complex influence of two opposed treatment effects, the one acting primarily at lower concentrations to cause the shifts described above, the other acting at higher concentrations to reverse them, and presumably also to cause the general depression in double exchange at 81.6 μM (see DISCUSSION). It also suggests an answer to the nature of the differences in double-exchange data between experiments I and Ia. This is

that, since the curvatures of the experimental curves are discordant when $DE_{1,2}$ appears in the ratio and concordant when it does not, a differential response to treatment in this segment is indicated as the major source of discrepancy.

DISCUSSION

The analysis of single-exchange and double-exchange progeny as separate groups has not been used in physiological studies of crossing over, to the authors' knowledge. Nevertheless, it has two advantages over conventional linkage analyses, *viz.* a reduction in the complexity of the data group, and the possibility of comparison of the analytical results of two groups. Thus it has been seen in these experiments that DE effects can be assessed in terms of coincident-exchange tendency, and that the groups differ in overall effect, with DE's showing a consistent trend over the treatment range, and SE's none.

The latter progeny dominate the recombination estimates, and hence some pessimism concerning the suitability of variance analysis of recombinations may be justified. Nevertheless, the analyses of experiments I and II lend confirmation to the highly significant effects reported by the authors in *Hordeum vulgare* and those indicated by unpublished data of DR. C. PERSON from *Ustilago nuda*.

Since nutrient selenium, whether as selenate or selenoaminoacids, is known to be incorporated into protein, the significant effects on recombination frequency mentioned above may well be a consequence of the structural alteration of proteins in which selenoaminoacids are incorporated. The sulfhydryl group of cysteine, the disulfide bond of cystine and the thioether bond of methionine, replaceable by selenohydryl- and by diselenide- and selenoether-bonds, respectively, (JÁUREGUI-ADELL, *ibid.*) have important roles in enzymatic reactivity and protein structure (CECIL and MCPHEE 1959). There appear to be two major possibilities, therefore, for this selenium effect: either it is mediated through a change in the activity of enzymes controlling the exchange process, or it involves a deformation of structural protein that, by virtue of a linear association with the DNA, is normally capable of initiating DNA exchanges.

The attempt made here to reach a decision concerning these alternatives is based on the general premise that the two mechanisms are associated with entirely different expectations for the intensity pattern of exchange along the chromosome. A change in enzyme activity, on the one hand, might be expected according to the "random hit" nature of this activity to have an entirely random effect. The resultant alteration in the expectation of exchange should, in other words, be the same for a constant unit of map distance, wherever it is situated. Deformations of chromosomal protein, on the other hand, might be expected to cause changes in the mechanics of synapsis, and thus to relate with a varying pattern of mass and inertial distributions along the chromosome. The definite trends in redistribution of double exchanges and to a lesser degree, those for single exchanges along the chromosome favor the latter mechanism.

The hypothesis that the effects in this study may be mediated through altered enzymes, moreover, is open to serious question when faced with the results of our

analyses of the effects of treatment on DE's for experiment I and Ia (RESULTS, section 4). Here it was shown in experiment I, for example, that with low concentrations of selenocystine a relatively constant tendency to (coincident) exchange in segment 1 was accompanied by a decreasing tendency in segments 2 and 3. Thus, in order to sustain the argument, it would be necessary to add the illogical condition that altered enzymes would in one segment maintain, and in others inhibit, exchange. Moreover, it would still remain necessary to invoke some change in the chromosome itself to explain the switch within a single segment from an increasing to a decreasing exchange tendency indicated by the change in slope of the RDE curves in Figure 4 from low ($1.63\text{ }\mu\text{M}$ – $5.2\text{ }\mu\text{M}$) to high concentrations ($16.3\text{ }\mu\text{M}$ – $81.6\text{ }\mu\text{M}$).

In contrast, the hypothesis that the effects are mediated by alterations in the structural protein of the chromosome appears to permit a rational explanation of our results. One form at least that this hypothesis might take, however, appears unacceptable. This is that the effects could be mediated through such proteins by virtue of a "coating" function, whereby they might control the accessibility of exchange-controlling enzymes to the DNA. Localized alterations at selenoamino-acid insertion sites might, with this interpretation, permit an increased enzyme penetration and hence the increased tendency to exchange (coincident or single) indicated for segment 1 at low treatment concentration. This argument fails, however, for the same reasons as the previous one; *viz.*, that it can support neither the requirements of segmental reversal nor those of segmental switching of exchange tendencies with treatment.

We therefore find it necessary to conclude that a hypothesis of induction of DNA exchange by associated chromosomal protein appears to provide the best explanation of our results, and to couch our rationale for it in terms of the process of exchange and the morphology of the chromosome. The process of exchange favored is that of breakage-and-reunion, with the reunion mechanism, though probably enzyme-controlled (cf., WHITEHOUSE 1965) remaining relatively neutral for the effects described here (cf., JÁUREQUI-ADELL *ibid.*, *re* relative effects of selenium incorporation on structural and metabolically active proteins).

Hence the exchange events discussed here are treated as breakage events. The euchromatic region y – f under study, and illustrated in Figure 5 includes a "free" end (segment 1), a midarm (segment 2), and an "internal" part (segment 3) separated from a heterochromatin block (Het) by a short segment (f – car). The assumption is made that the terminus lies free in the nucleoplasm, and the

FIGURE 5.—Approximate relations of map and salivary gland chromosome distances along the X-chromosome.

heterochromatin, being more condensed in meiotic prophase, and hence more heavily coiled, is relatively more fixed. It is to be expected, with these assumptions, that according to the hypothesis being examined, exchange would normally be attenuated toward the terminus due to its low inertia. Some suggestion that such is the case is provided by the gross comparison of genetic and cytological map lengths in Figure 5.

The gradual drop in exchange density for the total segment, suggested by the three DE_T curves of Figure 4, may be explained, according to the aforementioned hypothesis, by a general relaxation of chromosomal protein as a result of selenoaminoacid incorporation. Thus the shifts in the density distribution of exchanges would be interpreted as being the result of this general relaxation; in other words, the pattern of exchange density varies with the degree of relaxation, but is constant for any relaxation level.

There is some suggestion of a transient but considerable increase, at trace concentrations of selenoaminoacid in DE_T for the environmentally concurring experiments I, II, which would be difficult to reconcile with general relaxation. It might be suggested that, with trace amounts of selenoaminoacid incorporated, only negligible effects on protein relaxation would occur; the incorporation sites might at the same time represent "weak points" in the protein, with high localized potentials for inducing DNA breakage. Such an effect could, indeed, be one of the opposed treatment effects inferred from our analysis of the DE data.

The very slight drop in SE_T with dose (RESULTS, Section 4, text), accompanied by marked inflective reversals in the SE curves of Figure 2, may appear to present difficulties to the hypothesis. On the other hand, they may simply reflect the statistical complexity of this group, which could be expected to benefit in frequency from reduction in DE while itself suffering a deficit in favor of the no-exchange group. Thus, beyond the observation that treatment effects on SE and DE appear not to be correlated (i.e. SE_1 for experiment I drops most, while coincident exchange in segment 1 drops least of the three segments), the authors find the data for this group obscure.

An interesting point of the DE analysis is that segment 1 in experiments I and I-a shows the least adverse effect of low-concentration treatments on coincident exchange. Thus the effects may be related to the type of inertial effect suggested for this segment in the discussion of Figure 5.

SUMMARY

Crossing-over was studied over the major part of the *X* chromosome in testcross progeny of γ *cv* *v f* / + + + + females fed as larvae on media containing a logarithmic concentration series of selenocystine (control, 1.63, 5.22, 16.3, 81.6 μ M) or selenomethionine (1.63, 5.22, 16.3 μ M). Serial reduction in recombination was significant by analysis of variance in one of two experiments with selenocystine and in the selenomethionine experiment. Double-crossover progeny, studied separately, showed a gradual overall drop in total frequency with dose from 1.63 μ M to 81.6 μ M in all experiments. This was accompanied by appreciable

shifts in the relative densities of double-exchange events in the three segment pairs at intermediate concentrations, and a reverse shift to the $1.63 \mu\text{M}$ values at $81.6 \mu\text{M}$. Analysis of these shifts indicated that relative initial attenuation of coincident crossovers was always least in $\gamma\text{-}cv$; results were equivocal with regard to the relative attenuation in $cv\text{-}v$ and $v\text{-}f$. These results are interpreted according to the polaron-hybrid DNA model of WHITEHOUSE as supporting the hypothesis that chromosomal protein, linearly associated with the DNA, mechanically induces breakage in the latter. The treatment curves for single-exchange chromosomes show considerable evidence of treatment effects on single segments, with little effect on the frequency for the total segment. These effects, which do not show obvious correlation to patterns for coincident crossovers in the corresponding segments, and effects in the control to $1.63 \mu\text{M}$ range, have not been elucidated.

LITERATURE CITED

- BAILEY, N. T. J., 1961 *Mathematical Theory of Genetic Linkage*. Clarendon Press, Oxford.
- BRIDGES, C. B., and K. S. BREHME, 1944 The Mutants of *Drosophila melanogaster*. Publication 552. Carnegie Institute of Washington, Washington, D.C.
- CECIL, R., and J. R. MCPHEE, 1959 The sulfur chemistry of proteins. *Advan. Protein Chem.* **14**: 256-389.
- JÁUREGUI-ADELL, J., 1966 Selenium derivatives in proteins. *Advan. Protein Chem.*, **21**: 387-415.
- MATHER, K., 1965 *Statistical Analysis in Biology*. Methuen, London.
- NICKERSON, W. J., W. A. TABER, and G. FALCONE, 1956 Physiological bases of morphogenesis in fungi. 5. Effect of selenite and tellurite on cellular division of yeastlike fungi. *Can. J. Microbiol.* **2**: 575-584.
- PAINTER, Z. P., 1941 The chemistry and toxicity of selenium compounds, with special reference to the selenium problem. *Chem. Rev.* **28**: 179-213.
- STEFFENSEN, D., 1957 Dwarf *Drosophila* produced by ethylene diamine tetra-acetic acid: A relationship to chelation of zinc. *Nature* **180**: 390-391.
- WALKER, G. W. R., and K. TING, 1967 Effects of selenium on recombination in barley. *Can. J. Genet. Cytol.* **9**: 314-320.
- WHITEHOUSE, H. L. K., 1965 *Towards an Understanding of the Mechanism of Heredity*. Arnold, London.