THE EFFECTS OF COMBINED RADIATIONS ON CROSSING OVER IN *DROSOPHILA MELANOGASTER*¹

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IN attempts at an experimental approach to the problem of the mechanism of crossing over many modifying agents have been used: temperature (PLOUGH 1917), aging (BERGNER 1928), inversions (STEINBERG 1936), mineral content (BROWNING 1949), and radiation (MULLER 1925). As diverse as are these agents, they all have one effect in common. They modify the rate of crossing over in either the region of the kinetochore or the region very distal to it. In most cases there is observed a pattern of change which KIKKAWA (1934) called, "the proximal increase and compensatory distal decrease". This similarity of effect suggests a common path of action by all the agents; but few attempts to construct a unifying hypothesis have been presented. Three which deserve mention are: the mechanical hypothesis of KIKKAWA (1934), one involving coiling modifications by MATSUURA (1940), and one stressing the role of heterochromatic areas (SCHULTZ AND REDFIELD 1951). These have much in common, and offer a good starting point for a generalized hypothesis.

In recent experimental investigations, much emphasis has been laid upon the effect of radiations. WHITTINGHILL and his associates (WHITTINGHILL 1954; HINTON and WHITTINGHILL 1950) have done a thorough analysis of this aspect; but they have devoted most of their discussion to a consideration of the time at which crossing over occurs rather than the mechanism whereby the radiation affects the process. However, since much stress is put upon the role of recombination in the gonial cells, one has the feeling that these authors believe that the radiation acts to cause increase in the number of breakage points. Such a role of modifying agents is also apparent in the recent publication of LEVINE (1955), in which it is suggested that the correlation between mineral deficiency and crossing over increase can be explained by rupture of the chromosomal strands at points of calcium binding. Both agents (X-ray and mineral deficiency) are known to disintegrate the chromosome structure (STEFFENSEN 1955) and both increase crossing over in certain regions. Although there are a number of objections to this idea, it is a natural assumption and one which merits further investigation. It was the increased breakage hypothesis which these experiments were designed to test.

It has been demonstrated that following treatment with infrared radiation chromosomes of *Drosophila* are broken more frequently by exposure to X-rays (KAUFMANN, HOLLAENDER and GAY 1946). It was felt that this potentiating effect of the infrared might apply to a normal chromosome breakage process in the course of crossing over. A test of this idea was made with negative results (IVES, FENTON, YOST and LEVINE 1953), and it was concluded that the type of recombination

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exhibited by crossing over is quite different from that shown by radiation induced translocations. However, this does not apply to the idea that radiation increases crossing over by an increase in breakage. It was felt that although there is no increase in recombination with infrared alone (just as there is no increase in chromosome breakage with infrared alone), there might be a potentiating effect of the infrared when used as a combined radiation. If this proved true, it should then be possible to test this effect with other modifying agents and perhaps gain some knowledge of the suggested common pathway of action. Furthermore, a recent paper by SNOAD (1955), has indicated that infrared radiation modifies the number of chiasmata in *Tradescantia*. This made an investigation of the effect of infrared on crossing over imperative.

Recently acquired evidence that the effects of infrared treatment in promoting chromosome breakage in *Tradescantia* are more pronounced with X-radiation than with gamma radiation (SWANSON, personal communication) suggests that the breakage of chromosomes by infrared radiation is related to the ion density of the supplementary radiation. It seemed possible that the use of different ionizing radiations might facilitate interpretation of the data. If the results were to show a greater effect in the combined X-ray-infrared series than in the combined gamma ray-infrared series, they might indicate similar mechanism to be operating in the induction of crossing over and in chromosome breakage. For this reason the tests were made using both types of radiation.

MATERIALS AND METHODS

The stocks used in this experiment were an Oregon-R wild type, a second chromosome marker stock, *net b cn bw*, and a first chromosome marker stock, *y cl⁶ ras² f*. The wild type stock had been inbred for 130 generations by single pair brother-sister matings. The marker stocks were synthesized in this laboratory about 15 years ago and had been maintained in close-bred vial cultures with about ten pairs of parents in each generation. At the beginning of the study, each stock was shifted to half pint culture bottles and maintained thereafter with about 100 pairs of parents per generation.

Females tested for crossing over in these studies were heterozygous for the wild type and one or other mutant marker stock. They were backcrossed to the marker stock males immediately after treatment. Individual females were mated to two or more males in shell vials of corn meal-molasses-agar-yeast medium and were shaken to new shell vials every day in most cases. In one series, the flies were shaken every three days. The treated virgins were collected at one time, meaning that their age did not vary by more than ten hours; they were exposed to infrared radiation at approximately 24 hours of age. All cultures were raised at 25°C.

The infrared source and the method of exposure has been described previously (YOUNG, YOST, IVES and LEVINE 1953). After a 24 hour exposure to infrared, at 10°C, the flies were treated with either X or gamma rays. The X-rays were delivered from a General Electric industrial machine, operated at 80 kv and 10 ma. The gamma radiation was obtained from a 440 curie Co⁶⁰ slug. In both cases the total dose was 1400r, as measured with an appropriate Victoreen dosimeter. The intensity was

200r per minute in both cases. At all times, the control flies received exactly the same treatment as the experimental flies, with the exception of exposure to the radiation.

Analysis of the data was done by using the chi-square test. The data are lumped only where it is permissible. Analysis of reciprocal classes showed no significant differences in these classes. There was no indication of clusters of crossover types.

EXPERIMENTAL RESULTS

For the sake of brevity certain abbreviations have been used in the tables. They are as follows: C—control flies; X—X-rayed flies; γ —gamma rayed flies; IR+—flies exposed to infrared radiation as a pretreatment to the ionizing radiation; (vs C) the chi-square test is computed against the control values; (vs X)—the chi-square test is computed against the values from the X-rayed series.

Table 1 presents the data obtained when the flies were exposed to infrared and X-rays. In this series the flies were shaken every three days, the marker stock was *net b cn bw*. Since the regions *net-b* and *cn-bw* are so large, the data for crossing over in these cases are omitted. There was no significant change from normal (in either direction) shown by these regions. It is evident that only the heterochromatic region near the kinetochore is being tested by this stock. These data indicate that from about the fourth day on both X-rays and combined treatment increase the rate of crossing over near the kinetochore. However, there is no indication of an added

TABLE 1

Crossing over in young adult females exposed to infrared radiation and X-rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% crov. <i>b-cn</i>	χ^2 (vs C)	χ^2 (vs X)
1-3	C	797	4.0		
	X	1223	3.7	0.1636	
	IR + X	1240	4.8	0.5922	1.8028
4-6	C	1022	2.7		
	X	1774	4.7	6.7768***	
	IR + X	1739	5.2	9.2476***	0.3492
7-9	C	1041	3.1		
	X	1610	9.0	35.6986***	
	IR + X	1529	8.5	30.6969***	0.2427
10-12	C	1295	3.7		
	X	1520	8.3	25.4081***	
	IR + X	1739	7.9	22.6094***	0.1810
13-15	C	1227	5.1		
	X	1470	8.0	9.1764***	
	IR + X	1646	7.6	5.1324***	0.1512
16-18	C	1051	5.3		
	X	1217	6.5	1.3797	
	IR + X	1348	7.9	6.3415**	1.9677
19-61	C	939	6.1		
	X	975	8.7	4.9091*	
	IR + X	1082	7.9	2.7300	0.4093

* Significant at the 0.05 level.

** Significant at the 0.02 level.

*** Significant at the 0.01 level.

effect of the infrared pretreatment. It was felt that the three day laying period might obscure the effects of the infrared treatment, since it was of only 24 hours duration, and it is known that some agents show effects for only as long as the duration of the treatment (PLOUGH 1917). To test this a new series was set up with transfer of flies every day. These data are presented in table 2. In this table data from two runs are presented. Because of the inherent variation in the data it was felt that separate presentation of the results would be better. It can be seen that with only one exception the values tend to be higher for the treated series than the controls from the first day on. It can also be seen that from the fourth day on the results are completely consistent. However, there is no significant difference between the X-ray and the combined treatment which is consistent. Thus the combined

TABLE 2

Crossing over in young adult females exposed to infrared radiation and X-rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	Run 1				Run 2			
		No. flies	% cov. b-cn	χ^2 (vs C)	χ^2 (vs X)	No. flies	% cov. b-cn	χ^2 (vs C)	χ^2 (vs X)
1	C	454	4.8			262	3.4		
	X	322	6.5	0.9738		281	4.3	0.0324	
	IR + X	357	6.7	1.3512	0.0148	294	7.1	3.6789	2.1630
2	C	433	4.2			415	3.6		
	X	553	5.8	1.3681		400	2.5	0.8733	
	IR + X	567	5.8	1.3681	0.8729	377	5.0	0.9658	3.4427
3	C	502	4.0			391	3.1		
	X	525	4.0	—		383	6.3	9.5454***	
	IR + X	490	5.3	0.9338	0.9728	407	3.4	0.7059	8.1262***
4	C	549	5.3			432	3.7		
	X	651	6.8	1.1377		362	7.5	5.4280**	
	IR + X	528	8.3	4.0511*	1.0053	431	4.9	0.7059	2.3250
5	C	509	4.5			353	6.0		
	X	568	8.8	7.7969***		354	8.9	1.3774	
	IR + X	466	7.9	4.9030*	0.2454	421	11.9	8.1232***	2.8634
6	C	481	3.7			377	7.2		
	X	489	10.6	17.1774***		337	13.1	6.9190***	
	IR + X	555	11.5	21.5096***	1.8545	414	15.9	14.6205***	1.2554
7	C	473	6.3			382	8.6		
	X	553	12.5	11.0885***		373	13.9	5.3037*	
	IR + X	479	10.4	5.2428**	1.0727	445	12.1	2.6792	0.5982
8	C	397	7.0			298	6.7		
	X	505	10.3	2.8857		190	13.7	6.6246**	
	IR + X	466	9.2	1.3645	0.3159	336	15.5	12.1507***	0.2991
9	C	504	4.2						
	X	529	8.7	8.7444***					
	IR + X	493	6.1	1.9048	2.4802				
10	C	434	8.1						
	X	572	6.6	0.7376					
	IR + X	640	11.2	2.8994	7.7517***				

* Significant at the 0.05 level.

** Significant at the 0.02 level.

*** Significant at the 0.01 level.

treatment is significantly higher than the X-ray on the tenth day of testing in Run 1, and the X-ray is significantly higher than the combined treatment on the third day in Run 2; but there is no other occurrence of such a difference on the third day in the rest of the data and only one other suggestion of such a difference at the

TABLE 3

Crossing over in young adult females exposed to infrared radiation and gamma rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% cov. b-cn	χ^2 (vs C)	χ^2 (vs γ)
1-3	C	555	2.7		
	γ	480	5.0	3.7294	
	IR + γ	323	5.6	4.7197*	0.1265
4	C	385	3.4		
	γ	252	6.0	2.3858	
	IR + γ	171	6.4	2.6465	0.0426
5	C	289	3.5		
	γ	173	6.4	2.1877	
	IR + γ	141	13.5	15.0339***	4.5046*
6	C	246	6.1		
	γ	270	19.0	18.6006***	
	IR + γ	245	11.8	5.0339**	4.8187*
7	C	370	5.4		
	γ	383	14.1	15.9324***	
	IR + γ	269	17.5	24.1775***	1.3551
8	C	382	7.1		
	γ	470	16.6	17.7176***	
	IR + γ	338	14.5	10.4470***	0.6455
9	C	419	7.6		
	γ	540	13.9	9.3638***	
	IR + γ	335	13.7	7.3939***	0.0364
10	C	405	8.4		
	γ	587	10.7	1.4832	
	IR + γ	309	15.2	8.0310***	3.7967
11	C	331	7.8		
	γ	462	10.6	1.4779	
	IR + γ	279	10.8	1.5338	0.0241
12	C	349	9.2		
	γ	389	10.0	0.1600	
	IR + γ	200	9.5	0.0149	0.0418
13	C	351	10.8		
	γ	514	12.8	0.7995	
	IR + γ	268	10.4	0.0248	0.9470
14	C	354	11.6		
	γ	378	16.4	3.5036	
	IR + γ	157	9.6	0.9061	4.7659*
15-17	C	375	10.7		
	γ	550	12.0	0.3978	
	IR + γ	292	11.0	0.0155	0.2038

* Significant at the .05 level.

** Significant at the .02 level.

*** Significant at the .01 level.

tenth day (table 3). In general, when there are variations in the crossing over values between the two treated series, they tend to cancel each other in the two different runs.

Table 3 presents the data obtained with gamma ray treatment. The flies were shaken at the end of the third day and every day thereafter until the 15th day.

TABLE 4

Crossing over in young adult females exposed to infrared radiation and X-rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% crossover.			χ^2 <i>y-cl</i> region	
			<i>y-cl</i>	<i>cl-ras</i>	<i>ras-f</i>	(vs C)	(vs X)
1	C	192	16.7	11.5	27.1		
	X	423	17.0	9.2	25.5	0.0134	
	IR + X	166	22.9	7.8	28.9	0.0032	0.0024
2	C	499	15.8	12.0	24.0		
	X	652	16.6	10.1	26.4	0.1146	
	IR + X	667	19.8	13.2	25.2	3.0184	2.2903
3	C	574	17.1	12.7	26.1		
	X	691	21.1	10.6	25.6	3.0581	
	IR + X	739	16.4	9.6	25.6	0.1180	5.0344*
4	C	646	22.0	12.1	24.5		
	X	603	19.9	11.9	25.0	0.8162	
	IR + X	808	18.2	13.7	24.0	3.2359	0.6569
5	C	740	20.3	9.3	25.8		
	X	801	16.1	9.7	23.0	4.4886*	
	IR + X	853	15.4	10.4	25.4	6.5381**	0.1754
6	C	385	18.4	10.1	24.2		
	X	590	12.5	9.5	24.4	6.4537**	
	IR + X	485	11.1	8.9	29.1	9.3353***	0.4907
7	C	498	18.1	9.8	25.3		
	X	735	12.2	8.6	23.5	8.0868***	
	IR + X	532	13.3	8.3	20.9	4.3867*	0.3388
8	C	391	17.4	8.4	27.4		
	X	508	14.4	7.3	26.8	1.5368	
	IR + X	521	11.5	8.3	23.4	6.3668**	1.8931
9	C	495	17.4	10.5	25.9		
	X	484	12.0	8.9	22.7	5.6741**	
	IR + X	436	10.1	8.5	23.6	10.2548***	0.8182
10	C	353	16.4	7.9	24.9		
	X	568	13.6	8.8	22.0	1.4581	
	IR + X	372	12.4	10.8	22.3	2.4624	0.2855
11	C	400	19.5	11.5	21.2		
	X	548	13.7	11.3	25.7	5.7381**	
	IR + X	568	12.1	9.3	21.7	9.7827***	0.5902
12	C	398	15.1	11.1	24.6		
	X	607	13.7	9.4	21.6	0.3954	
	IR + X	438	12.1	13.2	24.9	1.5753	0.5555
All days combined	C	5571	18.2	10.6	25.1		
	X	7210	15.3	9.6	24.5	18.6160***	
	IR + X	6585	14.5	10.5	24.4	29.6036***	1.7804

* Significant at the .05 level.

** Significant at the .02 level.

*** Significant at the .01 level.

TABLE 5

Crossing over in young adult females exposed to infrared radiation and gamma rays. Dose: 24 hrs. IR; 1400r

Day	Treatment	No. flies	% Crossover			χ^2 y-cl region	
			y-cl	cl-ras	ras-f	(vs C)	(vs γ)
1	C	233	15.0	9.0	26.6		
	γ	104	19.2	11.5	25.0	0.9158	
	IR + γ	204	18.6	14.2	26.0	0.5589	0.0152
2	C	447	18.8	11.9	26.6		
	γ	292	17.5	13.4	26.0	0.2183	
	IR + γ	366	20.8	10.9	26.5	0.5030	1.1109
3	C	638	21.8	11.1	25.9		
	γ	558	18.3	11.1	27.1	2.3008	
	IR + γ	659	19.3	11.7	24.7	1.2716	0.1950
4	C	548	21.2	12.2	24.3		
	γ	442	19.9	9.0	23.1	0.2398	
	IR + γ	576	20.0	10.6	23.3	0.2530	0.0025
5	C	510	21.0	13.1	25.3		
	γ	337	20.5	9.2	21.7	0.0299	
	IR + γ	427	20.1	10.3	22.5	0.1052	0.0118
6	C	528	23.7	9.3	23.7		
	γ	329	20.1	11.6	21.6	1.5183	
	IR + γ	437	20.1	9.8	21.1	1.7554	0.0033
7	C	681	23.2	11.7	23.6		
	γ	557	13.4	11.7	26.2	18.9700***	
	IR + γ	412	13.1	9.0	23.5	16.7154***	0.0234
8	C	391	17.9	12.5	24.0		
	γ	426	14.3	9.9	20.9	1.9418	
	IR + γ	384	16.1	10.4	26.0	0.4237	0.5267
9	C	390	17.4	13.8	23.1		
	γ	410	15.1	9.3	24.1	0.7793	
	IR + γ	368	14.7	8.4	23.4	1.0532	0.0329
10	C	388	18.0	10.1	26.0		
	γ	410	14.4	10.7	24.1	1.9729	
	IR + γ	318	16.0	11.0	21.1	0.4915	0.3656
11	C	335	17.0	11.6	20.9		
	γ	481	16.0	12.5	24.7	0.0821	
	IR + γ	360	11.9	8.6	26.9	3.6216	2.7363
12	C	379	18.5	10.3	25.3		
	γ	330	15.2	10.3	27.9	1.4029	
	IR + γ	171	12.3	11.7	25.1	3.2755	0.7449
All days combined	C	5468	20.1	11.3	24.6		
	γ	4676	16.7	10.8	24.4	19.3529***	
	IR + γ	4682	17.4	10.4	24.0	11.8759***	0.8531

* Significant at the .05 level.

** Significant at the .02 level.

*** Significant at the .01 level.

The pattern of results is the same as that found with X-rays. Note that the increase in crossing over caused by combined treatment on the tenth day is *not* significantly different from that obtained with X-rays.

The data presented in tables 4 and 5 are those obtained with the same treatments

as above but with the first chromosome marker stock, $y\ ct^6\ ras^2\ f$. These experiments were undertaken to see if smaller euchromatic regions not associated with the kinetochore would show some effect of the infrared pretreatment. The data in table 4 were obtained using X-rays and those in table 5, with gamma rays. From the summary of all days tested, at the bottom of each table, it can be seen that there is little difference between the two groups. The results in table 5 show decreases in the $y\ ct$ region similar to those in table 4, but they are not significant in any case other than day seven. This difference in effect is due partly to the lower hatch in this experiment compared to the experiment presented in table 4. It seems unwise to conclude that there is any difference which can be attributed to the differential effects of gamma radiation on the basis of these few data.

Furthermore, it can be seen that in each case the reduction of crossing over is largely in the $y\ ct$ region. For these reasons, the discussion of the data will treat both tables as a unit and will be concerned with the distal region of chromosome one only. The change in crossing over follows the same general pattern shown by the $b\ cn$ region of chromosome two (although in opposite direction); that is, it is present, in some degree, by the fourth day and persists for an extended period thereafter. There is a slight indication of an increase in crossing over during the first day, but the numbers are small, and the increases are not significant.

DISCUSSION

There is a general tendency to consider that crossing over results from a process similar to translocation. This is the result of evidence of segmental interchange coincident with genetic exchange (STERN 1931; CREIGHTON and MCCLINTOCK 1931). Therefore, when agents which are known to increase translocation also increase crossing over, it is natural to ascribe the effect to an increase in chromosome breakage. In the work with radiations, this has been generally the case (see MULLER 1954; KAUFMANN 1954). However, there are some strong objections to attributing the modification of the rate of crossing over to increased chromosomal breakage. One of the most powerful is the *decrease* in crossing over, observed by many workers and reported in this paper, in the distal regions of the chromosome. MULLER (1954) has suggested that this decrease might either result from interference (due to increases in the proximal regions) or to irregularities of chromosome movement. These mechanisms seem unlikely, since interference of the usual type should affect the regions most strongly which are closest to the regions of increase, and since any alteration in mobility of the chromosomes should affect all regions equally. There can be little problem of synapsis, as the chromosomes are probably synapsed in the gonial stage in the Diptera.

A second objection to the breakage hypothesis as applied to radiation effects comes from the data of KAUFMANN (1941). It was shown that the breaks induced by X-radiation are distributed at random along the chromosomes of *Drosophila*, and that there is no tendency for the accumulation of breaks in heterochromatic regions. This would seem to contradict the breakage hypothesis, for the major effect of the radiations on crossing over induction is on regions of heterochromatin associated with the kinetochore. It may be argued that these regions are cytologically

large and that therefore any increase in breakage would show up as a pronounced effect upon genetic exchange. But this means that the radiation induced breakage is of a type that permits recombination in spite of the difficulties placed in its way by the heterochromatin, whereas "crossing over breakage" cannot. The difficulties with this argument become even greater when one considers that the heterochromatin is not completely limited to the region of the kinetochore and the major effect of the radiations is, particularly at low doses.

The above doubts are reinforced by a summary of other objections recently made by SAX (1954). "Unlike the chromosomal recombinations induced by radiation and certain chemical agents, the crossovers are always polarized and progressive from the proximal to the distal ends of the chromosome arms. Lateral fusions which give rise to dicentric bridges seldom if ever occur in normal meiosis. Yet the breakage and reunion of chromosomes induced by ionizing radiation show no evidence of polarity of the bonds between genic elements. An induced inversion is usually viable and functions as well as the original chromosome. These induced breaks, whether in sister chromatids or in different chromosomes, may also reunite laterally to give rise to dicentrics, or progressively to give rise to exchanges and translocations".

Furthermore, as the data in this paper show quite clearly, there is no enhancement of the effect of ionizing radiation when pretreated with infrared, although under these same conditions infrared will cause an increase in the number of translocations (KAUFMANN, HOLLAENDER and GAY 1946). It would appear that the infrared radiation is incapable of modifying the effects of ionizing radiation or the normal crossing over process (IVES, FENTON, YOST and LEVINE 1953). Some objection may be raised to these data in that the temperature of treatment was not quite so low as that reported by OSTER (1954). A test of this temperature effect is now under way at this laboratory. However, it must be kept in mind that the increased induction of translocations occurs with great magnitude at temperatures of 18°C or lower (KAUFMANN, HOLLAENDER and GAY 1946); therefore the major objection based on these data to the breakage hypothesis stands.

Finally, one must consider that many agents which influence crossing over (temperature, aging, inversions) are not generally considered to be agents which increase chromosome breakage. Nevertheless, in all these cases the pattern of change is the same. When one considers that in all cases of modification of crossing over the pronounced effect is always in the same regions (the area adjacent to the kinetochore or the region most distal to it), and when one considers that in most cases the effect is an increase at the kinetochore and a decrease at the distal ends, the conclusion that these various agents have a common pathway in the cell becomes inevitable. When one considers the evidence presented in this paper, coupled with the arguments from most of the work done in this field, the idea that the common pathway is chromosome breakage becomes most unlikely.

In like manner, many of the arguments presented will apply to the hypothesis that induced crossing over is the result of exchanges which occur in gonial cells (WHITTINGHILL 1954). Although the evidence is irrefutable that gonial crossing over does occur, and that in certain instances the majority of induced crossing over (even in females) can be interpreted in this way (HINTON and WHITTINGHILL 1950), the

extrapolation of the hypothesis to include all induced changes is unwarranted. Gonial exchanges could account for increases in the region of the kinetochore, but how would they account for the distal *decrease* which is compensatory to it? In some cases the effect appears too soon to be a gonial event (PLOUGH 1917). There can be no doubt that when a suppressor of normal crossing over is used (WHITTINGHILL 1954) the induced crossing over is largely gonial; however, under these conditions only that crossing over which is of a translocation nature would be expected, all other crossing over being suppressed. In view of the failure of the gonial exchange mechanism to explain the decreases observed in some regions, it would seem likely that the effect is upon the normal meiotic process, with some contribution (depending upon the agent and the situation) from gonial exchanges. Therefore, it appears that it is necessary to look for a mechanism which would permit common action by a variety of agents, and a mechanism which permits an effect of long duration. This last restriction is necessary as there can be no doubt that most of the chromosomes which exhibit the results of modified crossing over are treated in the gonial state (PLOUGH, 1924).

One mechanism which presents itself for the explanation of the induced modifications in crossing over is alteration of the coiling of the chromosomes. MATSUURA (1940) and SCHULTZ and REDFIELD (1951) have reviewed much of the evidence for this point of view. Their arguments will not be repeated here; however, some extensions may be worth while. It has been demonstrated by MATSUURA (1940) and SWANSON (1943) that the coiling of meiotic chromosomes is sensitive to both low and high temperatures. Thus relatively low amounts of energy input will produce gross changes in the structure of the chromosome. Since the chromosomes are probably in a partially coiled state at all times (the large coils of metaphase being exaggerations of a coiling at the polymer level (SAX 1954)), the genetic map distance must vary with the state of the coiling pattern at all times. Since the map distances do not vary greatly from one time to another, it seems likely that the pattern of coiling (at any level) is determined with a fair degree of exactness. On this basis, we might say that the region of the kinetochore (or heterochromatin in general) (WELSHONS, 1955), is more tightly coiled than the other regions and thus exhibits a low frequency of crossing over. Any agent which could cause a relaxation of the coiling in this region would cause an increase in crossing over, and similarly if the amount of coiling is predetermined, there might be a compensatory change in the pattern distal to the kinetochore and a reduction in the number of crossover points. Thus we may make the hypothesis that agents which modify the rate of crossing over do so by an alteration of the normal pattern of coiling of the chromosome.

It is necessary to consider two things about this hypothesis. How is the degree of coiling modified, and what is meant by *coiling* in this sense? These questions can be partially answered at this time. When we speak of the chromosome, we speak of a large body composed of many molecules held together by an almost numberless set of crosslinkages. The nature of these crosslinkages is not completely clear; but there are some which are usually considered to play and which undoubtedly do play a great role in binding protein molecules together (S—S; peptide; salt linkages; etc.), others which have recently been demonstrated necessary for the joining of macro-

molecular fragments (MAZIA 1954) (bonds which involve, or are sensitive to, divalent cations), and others which probably are available to a nucleoprotein (STEFFENSEN 1955) (phosphate bridges, with or without the mediation of cations). It is known that large molecules of a polymer nature tend to fold up (taking the form of a helix, or a folded chain) as crosslinkages are formed. The greater the number of linkages the greater the degree of folding. Thus as a protein molecule coils it is bringing about more possibilities of crosslinkages, and if they are available a tightly folded molecule will result. If one permits the substitution of the words polymer, or macromolecular particle, for the word, molecule, in the preceding sentence, it is possible to consider the coiled state of the chromosome to be a reflection of the degree of "coiling" of the polymers composing it. In this loose manner one may define what is meant by coiling in the sense applied to the problem of crossing over. That is, a tightly coiled area is one which through the formation of a considerable number of linkages (or very strong linkages) has reduced the effective distance between genes, either by making pairing impossible (the genetic material being on opposite sides of the coiled strands), or by reducing the linear distance between genes.

The other question may now be answered. The effect of the agents is (directly or indirectly) upon the linkages between or within the macromolecules of the chromosome, which permit coiling to take place. A suggestion of this mechanism comes from the work on the effect of mineral changes on crossing over (BROWNING 1949; LEVINE 1955). This work indicates that agents which remove important divalent cations are able to modify the crossing over process. BROWNING (1949) observed the same compensatory behavior which is found with other agents. Therefore one is tempted to think that the effect of all the other agents is upon linkages which are cation sensitive. A recent suggestion that the chromosome contains cross linkages involving phosphate and Ca (STEFFENSEN 1955), presents an interesting possibility for the partial explanation of the observed results with induced crossing over. It is possible that the coiling of the chromosome is dependent upon cation-phosphate complexes similar to those upon which muscle action is dependent. In this case, either through direct binding of the elements by the complex or by the action of the electrostatic forces involved (MORALES, BOTTS, BLUM and HILL 1955), the degree of coiling would depend upon the amount of complexing material available and the ability of the various binding sites to compete for the material. We might then predict that removal of the material from one region of the chromosome (by any one of a number of methods) would free it to be taken up at another region, resulting in a "compensatory" change in the coiling of these two regions. The best part of such a mechanism is that it can be modified in so many different ways to produce the same general result when observed as a change in the rate of crossing over. Thus modifications can be made in the phosphate pool available to the nucleus, or in the cation concentration, or in the number, or the affinity, of the binding sites. The effects could be either directly upon the chromosome or upon the general metabolism; they might be of temporary or of long range consequence; or they might be a combination of all these things, with one factor becoming more dominant depending upon the duration of the treatment or of the amount of energy input.

It is unnecessary to carry this treatment further. With two independent variables

almost any observed result can be explained. Indeed, this hypothesis can be expanded to interpret all the observed effects of various agents upon crossing over.

It might be relevant to mention that this hypothesis permits an explanation of the interchromosomal effects observed with inversions. An inversion interferes with the normal synaptic process and causes a disarrangement of the normal coiling pattern of the inverted chromosome and its partner. This results in a change in the availability of the complexing material and thus a change in the coiling of the other chromosomes. The fact that the degree of the effect shows no direct relation to the size of the inversion (STEINBERG and FRASER 1944) is to be expected in this case, as the determining factor should be the degree of coiling normally shown by the inverted area. Furthermore this hypothesis has the advantage that the number of chiasmata need not be limited (MATHER 1936), since a change in the complex pool will alter the total map distance so that a net increase in crossing over can occur; and it will apply to cases in which non-homologous pairing (SCHULTZ and REDFIELD 1951) can not be demonstrated.

It is important to note the following reservations. First, this is not a mechanism to explain normal crossing over. It can be expanded into such a mechanism, either by the assumption that in normal crossing over segmental interchange occurs as a result of coiling tensions within the chromosome (MATSUURA 1940) or as a result of normal dissociations in the complex-bound segments of the chromosome (MAZIA 1954). However, since a nonbreakage mechanism has some strong attractions (BELLING 1933; SCHULTZ and REDFIELD 1951) it seems wise to be cautious at this level. Secondly, it is not meant to rule out the participation of "translocation-type" exchanges in induced crossing over. It is probable that with ionizing radiations and with mineral deficiency there will be some induction of exchanges. However, we must expect many of these induced breaks to form illegitimate fusions and thus to be lost. This is particularly true of cells treated in gonial stages in which a division intervenes between treatment and division. The final product observed must be a mixture of normal crossing over, induced exchanges, and induced increases in the rate of crossing over through normal methods. For the reasons presented above, it is felt that the major contribution of modifying agents is in the last form. Thirdly, it must be borne in mind that this hypothesis is put forward to explain the results obtained in *Drosophila*, in which somatic synapsis plays an important role. The state of coiling will be of importance in such an organism at any stage. There is some doubt that induced crossing over would follow the same pattern in other organisms which do not show such pairing.

Finally, it must be pointed out that neither the data presented here nor those presented by IVES, *et al* (1953) are in agreement with the data of SNOAD (1955), which indicate an increase in the number of chiasmata with infrared treatment. This may be due to the difference in the organisms. *Tradescantia* and *Drosophila* do not always respond in the same way, and in the case of infrared there seems to be a temperature difference for the infrared effect (OSTER 1954). On the other hand, one might assume that in this case there is further evidence that genetic crossing over and chiasmata formation do not correspond exactly. Until studies are presented which can demonstrate an increase in chiasmata without a concomitant increase in

crossing over in one organism no further conclusions can be drawn. However, it is interesting to note that the change in chiasmata formation follows the same pattern found with changes in crossing over induced by other agents.

SUMMARY

Data are presented which show that infrared radiation delivered for 24 hours at 10°C is unable to modify the effect of ionizing radiations on the induction of crossing over. These data are considered in the light of other work on induced crossing over, and it is concluded that chromosome breakage is an unlikely mechanism to explain induced crossing over. The problem of induced crossing over is considered in the light of an hypothesis that the effect is upon the coiling pattern of the chromosomes. It is suggested that the use of infrared radiation may be one way to determine the correspondence between chiasmata formation and genetic crossing over.

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