THE PHENOTYPES OF HOMOZYGOTES AND HEMIZYGOTES OF POSITION ALLELES AND OF HETEROZYGOTES BETWEEN ALLELES IN NORMAL AND TRANSLOCATED POSITIONS¹

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Received June 17, 1946

EXCEPT for rare cases of additive expression, it is a rule which is nearly without exception that the heterozygote between two alleles is phenotypically either identical with one of the two homozygotes or intermediate between them. In experiments involving position effects of the fourth chromosome normal allele of cubitus interruptus (*ci*) of *Drosophila melanogaster*, several cases have been found where this rule does not hold but where the heterozygotes lie phenotypically outside of the range delimited by the homozygotes.

MATERIAL AND METHODS

DUBININ and SIDOROW (1934) showed that the dominance of the normal, + allele over its recessive allele *ci* is frequently weakened when a translocation occurs near the *ci* locus. To gain additional data on the nature of this effect, males of an isogenic wild type stock (Canton-S) were irradiated with a 4000r dose of X-rays and mated to females homozygous for *ci ey^R*. The offspring were all heterozygous +/*ci ey^R* and most of them were normal phenotypically (*ey^R* = allele of eyeless recessive chromosome 4). A small number, however, showed various degrees of interruption of the fourth vein. Nineteen such individuals and the stocks derived from them were analyzed (STERN, SCHAEFFER, and HEIDENTHAL 1946). All proved to contain "position alleles," *R*(+), defined as rearrangements ("*R*") involving the neighborhood of the + allele at the *ci* locus.

Crosses were made between *ci ey^R/ci ey^R* females and *R*(+)/*ci ey^R* males of all 19 different *R*(+) stocks. The offspring of these crosses consisted of round eyed *R*(+)/*ci ey^R* and small eyed *ci ey^R/ci ey^R* sibs, disregarding some additional individuals due to unbalanced genotypes and to nondisjunction. The flies were classified as to degree of interruption of veins by arbitrarily distinguishing five classes—0, 1, 2, 3, 4—from no interruption to complete absence of the vein. (This designation of the five phenotypic classes replaces the designation N, 0, 1, 2, 3 prior to 1946.) An index of expressivity was then calculated by giving each class the weight of its class designation 0–4 and determining the mean of the distribution. Separate means for each culture were obtained for females and males of each of the two genotypes *R*(+)/*ci ey^R* and *ci ey^R/ci ey^R*, and total mean values for each sex and genotype were deter-

¹ Supported in part by a grant from THE ROCKEFELLER FOUNDATION.

² Deceased August 2, 1945.

mined from the sum of the distributions of all cultures of each $R(+)$ allele. These values, for the females, lie between 1.03 and 3.44 for $R(+)/ci\ ey^R$ and between 2.00 and 2.84 for $ci\ ey^R/ci\ ey^R$; for the males, between 0.96 and 3.80 for $R(+)/ci\ ey^R$ and between 2.34 and 2.97 for $ci\ ey^R/ci\ ey^R$ (see STERN, SCHAEFFER, and HEIDENTHAL 1946, table 1).

The different degrees of expressivity are the result both of intrinsic differences between the different $R(+)$ alleles and of environmental differences influencing the degree of vein interruption. Since environmental variations

TABLE 1

Distribution of differences between the means of expressivity of $ci\ ey^R$ flies and the means of nineteen different $R(+)/ci\ ey^R$ heterozygotes. In all cases except $R^{15}(+)$ (see special treatment) differences are based on comparisons of sibs.

DIFFERENCES BETWEEN MEANS OF $ci\ ey^R/ci\ ey^R$ AND $R(+)/ci\ ey^R$.							
		> 1.50	1.00-1.49	0.50-0.99	0.0-0.49	-(0.01-0.49)	-(0.50-0.99)
Stock number of $R(+)$ allele	♀ ♀	11	19, 20	1, 5, 6, 8, 10, 14	16, 18, 21	7, 9, 12, 13	2, 3, 15
Total	19	1	2	6	3	4	3
Stock number of $R(+)$ allele	♂ ♂	11	1, 5, 6, 10, 14, 20	8, 16, 18, 19	7, 9, 13, 21	12, 15	2, 3
Total	19	1	6	4	4	2	2

should affect $R(+)/ci\ ey^R$ and $ci\ ey^R/ci\ ey^R$ flies similarly, the influence of environment can be largely excluded if the $R(+)$ alleles are not characterized simply by their values of expressivity but rather by the differences between the expressivity values of the $ci\ ey^R/ci\ ey^R$ individuals and their like-sexed $R(+)/ci\ ey^R$ sibs. These difference values are given in table 1. A positive difference means that the heterozygote is less abnormal than the $ci\ ey^R/ci\ ey^R$ homozygote, while a negative difference signifies the opposite. It is seen that seven $R(+)$ alleles give negative differences for females and four for males. The $R(+)$ alleles giving negative differences for males are included among those giving negative female differences. In other words, four $R+$ alleles, numbered $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$ in chronological order of their initial discovery, produce in both sexes, as heterozygotes, more extreme interruption than $ci\ ey^R/ci\ ey^R$ sibs, while $R^7(+)$, $R^9(+)$, and $R^{13}(+)$ are more extreme than $ci\ ey^R/ci\ ey^R$ in females only, but less extreme in males. The remaining 12 $R(+)$ alleles give positive differences for both sexes.

$R^7(+)$, $R^9(+)$, and $R^{13}(+)$ heterozygotes are only slightly different from the $ci\ ey^R/ci\ ey^R$ homozygotes so that the fact of positive differences for the males and negative ones for the females may represent only chance deviations from a true value close to zero. Statistically the negative differences for $R^9(+)$ and $R^{13}(+)$ females are not significantly different from zero.

In the following an analysis of the position-alleles $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$ is presented. Of these, $R^3(+)$, which is a more complicated rearrangement than the others, will be treated last.

CYTOLOGICAL DETERMINATIONS

$R^2(+)$. Translocation between the right arm of chromosome 3 ("3R"), broken at the very end of section 82D (BRIDGES map, 1935) and chromosome 4, left arm. The break in 4L is not recognizable in salivary gland nuclei.

FIGURE 1.—Diagram of the $R^3(+)$ rearrangement.—A. Normal chromosomes 2, 3, and 4 (the latter split into two chromatids). Position of breakage points are indicated.—B. The rearranged chromosome elements.

Oogonial metaphases show a rod shaped chromosome considered to be 3L and a very unequal armed chromosome considered to be 3R and 4R.

$R^{12}(+)$. Translocation between 3R and 4R. Break in 3R immediately following 98F1; in 4R in region 101 very close to the kinetochore. Oogonial metaphases of flies heterozygous for the translocation appear nearly normal with one of the two spherical chromosomes slightly smaller and more diffusely stained than the other. Presumably the diffuse element corresponds to the base of chromosome 4 and the tip of 3R attached to it.

$R^{15}(+)$. Insertion of a section of 4R into 2L. Breaks in 4 immediately before 101F1 and immediately after 102C4. Inserted in 30A between the first and last

heavy band—that is, inside the capsule. Oogonial metaphases of flies heterozygous for the translocation appear normal except for one of the two spherical chromosomes slightly smaller than the other. The smaller chromosome is the deficient one.

$R^3(+)$. A complex rearrangement, involving at least six breaks (see fig. 1A). (1) in 2L to the right of 21D1; (2) and (3) in 3L to the right of 67C2 and to the left of 75A1 respectively; (4) and (5) in 3R to the left of 95D/E and of 97C2 respectively; and (6) in 4R to the left of 101F1, very close to the kinetochore. In the rearrangement the nearly complete right arm of 4 has become translocated at the 67C2 end to the segment 67C2–75A1 of 3L, which in turn has become attached with its 75A1 end to the 21D1 breakage point of 2L. The whole rearranged sequence is shown in fig. 1B.

It is seen that the short section 95D/E–97C1 of 3R is not a part of the rearranged sequence. This section occurs as an independent chromosome unit (KODANI and STERN 1946). In salivary gland nuclei its 95D/E end is regularly embedded in the chromocenter. Frequently the 97C1 end is also joined to the chromocenter so that the section 95D/E–97C1 forms a short loop. Presumably the proximal end of the fragment is translocated to a base of chromosome 4 which thus would be present in addition to the other base of 4 which carries the translocated tip of 3L. It is possible that the two bases of 4 originated as a result of breaks induced in the two chromatids of chromosome 4 transmitted by the irradiated sperm. It is not clear how the 97C1 end of the excised section terminates. Its tendency to synapse with the chromocenter suggests the possibility that the section became inserted in a chromatid of 4 which itself became deleted of nearly all of 4R excepting the base and very tip.

Oogonial metaphases of flies heterozygous for the rearrangement possess normal X chromosomes, one normal chromosome each of 2, 3, and 4, and in addition a chromosome whose one arm is longer than a normal arm, another chromosome whose one arm is shorter than a normal arm, and a short rod-shaped chromosome two to three times longer than a normal chromosome 4. Obviously the unusually long arm represents the main part of 2L with parts of 3L and 4R added and the unusually short arm the base of 3L with the short tip of 2L attached to it. The rod shaped chromosome should be either made up of the base of 4 and the translocated tip of 3L, or of the base of 4 and the translocated excision from 3R. Either interpretation involves the assumption that one of the two elements, be it base of 4 and 3R fragment, or base of 4 and 3L tip are too small to be visible in oogonial preparations. Considering the relative length of the 3R and 3L fragments in the salivary chromosomes, it would be expected that the short section 95D/E–97C1 of 3R would be the one invisible. This was proven by simultaneous analysis of ganglionic metaphase chromosomes and of salivary gland chromosomes of certain larvae (KODANI and STERN 1946).

HEMIZYGOTES

It had been found by DUBININ and SIDOROW (1934) that the four different $R(+)$ alleles which they investigated in hemizygous condition led in this state

to normal, not interrupted vein phenotypes. This seemed surprising in view of the fact that the same $R(+)$ alleles in heterozygous combination with ci behaved like mutant ci alleles themselves.

Similar results were obtained with the four $R(+)$ alleles of the present study (table 2). Instead of using haplo-4 flies as hemizygotes, as the Russian workers did, the hemizygotes of $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$ contained the Minute-4 ($M-4$) carrying chromosome 4 which is deficient for a region including the ci locus. Most of the hemizygotes were obtained in the offspring of crosses of $R(+)/ci\ ey^R$ by $M-4/+$ parents. The resulting $M-4$ flies were of the con-

TABLE 2
Hemizygotes for $R(+)$ alleles.

GENOTYPE	TEMP.	PHENOTYPIC CLASSES			
		♀ ♀		♂ ♂	
		0	1	0	1
$R^2(+)/M-4$	14°-16°	65	—	76	—
$R^3(+)/M-4$	14°-16°	21	2	27	1
	26°	31	—	27	—
$R^{12}(+)/M-4$	16°	13	—	3	—
	26°	2	—	5	1
$R^{15}(+)/M-4$	15°-16°	62	2	51	8
	26°	22	1	20	—

stitutions $M-4/ci\ ey^R$ and $M-4/R(+)$. The former are known to possess wing interruptions nearly exclusively of the extreme classes 3 and 4. The presence of normal (class 0) or nearly normal (class 1) individuals was taken as proof of the presence of $M-4/R(+)$ individuals. There was a possibility that some of the Minute flies classified as $M-4/R^{15}(+)$ were in reality $Df_4[R^{15}(+)]/+$, where $Df_4[R^{15}(+)]$ signifies the deficient chromosome 4 of $R^{15}(+)$ and $+$ the normal chromosome 4 of the $M-4/+$ parent. A similar possibility existed in case of the $R^3(+)$ experiments where haplo-4 individuals might have originated in consequence of nondisjunction, thus obtaining no chromosome 4 from the $R^3(+)$ parent and a chromosome 4 with the normal allele from the $M-4/+$ parent. In order to exclude these sources of error which were present in the tests carried out at 15°-16°, the experiment was modified by using $M-4/ey^D$ instead of $M-4/+$ parents and inspecting the $M-4$, not ey^D offspring only (ey^D =eyeless-dominant, chromosome 4). This latter method or similar ones were adopted for all experiments carried out at 26°C. Their outcome did not differ essentially from that of the earlier experiments.

At the usual culture temperatures of 25°-26° the hemizygotes, with two exceptions only, were phenotypically normal notwithstanding the fact that the $R(+)$ alleles tested are unusually effective in producing extreme ci phenotypes in heterozygotes with ci . At lower temperatures, where the degree of interrup-

tion, if any, is known to be shifted toward greater expressivity, several $M-4/R^3(+)$ and $M-4/R^{15}(+)$ individuals of vein abnormality class 1 appear. While it is difficult to be certain that the frequency of these abnormal $M-4/R(+)$ individuals is greater than expected in nonposition allele $M-4/+$ flies, comparisons with earlier data on $M-4/+$ (STERN and SCHAEFFER 1943b) suggest that this is the case. This would indicate that the position-alleles $R^3(+)$ and $R^{15}(+)$ are slightly less effective in producing a normal phenotype than the $+$ allele in its unchanged locus.

HOMOZYGOTES

Of the four rearrangements, $R^3(+)/R^3(+)$ and $R^{12}(+)/R^{12}(+)$ proved to be inviable. Homozygous $R^2(+)/R^2(+)$ and $R^{15}(+)/R^{15}(+)$ survive, and stocks

TABLE 3

$R^2(+)$ homozygotes.

♀ ♀					♂ ♂				
0	1	2	3	4	0	1	2	3	4
4	1	58	33	—	1	1	45	35	—

TABLE 4

$R^2(+)$ homozygotes and $ci\ ey^R/ci\ ey^R$ raised in the same cultures.

$R^2(+)/R^2(+)$										$ci\ ey^R/ci\ ey^R$									
♀ ♀					♂ ♂					♀ ♀					♂ ♂				
0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
—	8	29	8	—	—	4	21	12	—	—	42	222	83	—	—	19	178	333	19

were established which were proved to be homozygous both genetically and cytologically.

In conformity with the observations of DUBININ and SIDOROW on three of their $R(+)$ alleles in homozygous condition, $R^{15}(+)/R^{15}(+)$ proved to be completely normal (203 ♀ ♀, 184 ♂ ♂).

Homozygotes for $R^2(+)$ are not normal but show interruptions of venation very similar in expressivity to those of homozygous mutant ci/ci (table 3). In order to compare the expressivity of $R^2(+)/R^2(+)$ and ci/ci under as similar conditions as possible, females of the former genotype inseminated by males of the same constitution and $ci\ ey^R/ci\ ey^R$ females inseminated by $ci\ ey^R/ci\ ey^R$ males were placed together in the same culture bottles and their offspring classified for degree of interruption of veins (table 4). It is seen that $R^2(+)$ homozygotes are slightly less extreme than $ci\ ey^R$ homozygotes. The difference in expressivity is significant for the males only.

The behavior of $R^2(+)$ homozygotes shows that the findings of DUBININ

and SIDOROW as to normality of homozygotes of $R(+)$ position alleles at the ci locus cannot be generalized. Of the total of five types of homozygotes described by DUBININ and SIDOROW and by the present authors, four exhibit the normal and one a mutant phenotype.

HETEROZYGOTES

The heterozygotes between any one of the four $R(+)$ alleles and the normal allele in the Canton-Special stock, $+^C$, were phenotypically normal, as shown by the normal venation of all but one offspring from crosses of $R(+)/ci\ ey^R \times +^C/+^C$. Half of the flies must have been heterozygous for $R(+)$ and $+^C$. The numbers observed in the four types of crosses were: $R^2(+)$, 233 ♀ ♀, 233 ♂ ♂ (one of these showed venation class 1); $R^3(+)$, 145 ♀ ♀, 171 ♂ ♂; $R^{12}(+)$, 153 ♀ ♀, 141 ♂ ♂; $R^{15}(+)$, 203 ♀ ♀, 184 ♂ ♂.

More interesting are the heterozygotes between the $R(+)$ alleles and ci as obtained from crosses of $ci\ ey^R/ci\ ey^R$ ♀ ♀ $\times$ $R(+)/ci\ ey^R$ ♂ ♂. The data will be presented in detail in order to enable the reader to form his own judgment as to the interpretation of phenotypes. It will be noted that different cultures of the same genotypes sometimes show phenotypic distributions which are different beyond chance expectations. On the whole, however, the homogeneity of the data in the face of the delicate response of ci venation to various environmental and genetic modifiers is striking.

$R^2(+)/ci\ ey^R$

The expressivity of this genotype can be judged from five separate cultures (table 5). The mean values for the expressivity of the $R^2(+)/ci\ ey^R$ flies of both

TABLE 5

F_1 of five separate cultures of 2 $ci\ ey^R/ci\ ey^R$ ♀ ♀ $\times$ 1 $R^2(+)/ci\ ey^R$ ♂.

CULTURE NUMBER	$R^2(+)$										$ci\ ey^R$									
	♀ ♀					♂ ♂					♀ ♀					♂ ♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	—	—	7	50	7	—	—	2	50	11	2	8	31	15	—	—	7	22	28	—
2	—	—	10	38	6	—	—	1	52	14	—	10	25	18	2	—	—	22	40	2
3	—	—	14	39	3	—	—	2	60	13	1	10	21	15	1	—	5	23	36	1
4	—	—	3	40	8	—	—	2	38	10	—	3	23	13	4	—	1	9	36	4
5	—	4	13	32	1	—	—	4	37	3	2	2	23	12	—	—	—	20	15	3
Total	—	4	47	199	25	—	—	11	237	51	5	33	123	73	7	—	13	96	155	10

sexes and their $ci\ ey^R/ci\ ey^R$ sibs, calculated separately for each culture and for the total of all, are listed in table 6.

The differences in mean values between $R^2(+)/ci\ ey^R$ and $ci\ ey^R/ci\ ey^R$ for the females vary between -0.45 and -0.95 with an average difference of -0.71 . For the males they vary between -0.30 and -0.77 with an average difference of -0.64 . In every case the $R^2(+)/ci\ ey^R$ value is greater than the corresponding $ci\ ey^R/ci\ ey^R$ value, showing that the heterozygotes are more

TABLE 6

Means of expressivity of $R^{12}(+)$ heterozygotes and $ci\ ey^R$ sibs listed in table 5.

CULTURE NUMBER	$R^{12}(+)$		$ci\ ey^R$		DIFF. BETWEEN MEANS	
	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂
1	3.00	3.14	2.05	2.37	-0.95	-0.77
2	2.93	3.19	2.22	2.69	-0.71	-0.50
3	2.80	3.15	2.10	2.51	-0.70	-0.64
4	3.10	3.16	2.42	2.86	-0.68	-0.30
5	2.60	2.98	2.15	2.55	-0.45	-0.43
Total	2.89	3.13	2.18	2.59	-0.71	-0.64

extreme than the mutant homozygotes. Since the latter are themselves slightly more extreme than $R^{12}(+)/R^{12}(+)$, the $R^{12}(+)/ci\ ey^R$ heterozygotes are more abnormal than either homozygote.

 $R^{12}(+)/ci\ ey^R$

The original data for four cultures and the mean values of expressivity are listed in tables 7 and 8. The values for $R^{12}(+)/ci\ ey^R$ females vary from 2.21

TABLE 7

 F_1 of four separate cultures of $2\ ci\ ey^R/ci\ ey^R$ ♀ ♀ $\times$ $1\ R^{12}(+)/ci\ ey^R$ ♂.

CULTURE NUMBER	$R^{12}(+)$										$ci\ ey^R$									
	♀ ♀					♂ ♂					♀ ♀					♂ ♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	2	3	8	30	1	—	2	3	23	—	4	25	25	2	—	1	16	22	—	—
2	—	8	5	16	3	—	1	4	23	1	—	3	11	5	—	—	3	9	9	—
3	—	8	10	29	2	—	5	9	20	2	—	12	27	23	3	—	2	12	26	2
4	3	11	7	25	1	—	1	6	22	—	—	15	21	11	—	—	1	10	19	2
Total	5	30	30	100	7	—	9	22	88	3	—	34	84	61	5	—	7	47	76	4

TABLE 8

Means of expressivity of $R^{12}(+)$ heterozygotes and $ci\ ey^R$ sibs listed in table 7.

CULTURE NUMBER	$R^{12}(+)$		$ci\ ey^R$		DIFF. BETWEEN MEANS	
	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂
1	2.57	2.75	2.42	2.54	-0.15	-0.21
2	2.44	2.83	2.11	2.29	-0.33	-0.54
3	2.51	2.53	2.26	2.67	-0.25	+0.14
4	2.21	2.72	1.91	2.69	-0.30	-0.03
Total	2.43	2.70	2.20	2.57	-0.23	-0.13

to 2.57, with an average of 2.43, for $ci\ ey^R/ci\ ey^R$ females from 1.91 to 2.42, with an average of 2.20. The male values vary from 2.53 to 2.83, average 2.70, and from 2.29 to 2.69, average, 2.57, respectively. In seven out of eight comparisons of $R^{12}(+)$ heterozygotes with $ci\ ey^R$ homozygotes the mean expressivity of $R^{12}(+)/ci\ ey^R$ is larger than that of $ci\ ey^R/ci\ ey^R$ sibs. In the one comparison which gives an opposite result the difference is statistically not significant. The average difference for the females is -0.23 , for the males -0.13 . Both these values, though small, are significantly different from zero.

$R^{15}(+)/ci\ ey^R$

The cross of $R^{15}(+)/ci\ ey^R \times ci\ ey^R/ci\ ey^R$ results, among both sexes, in the following four different viable genotypes: (1) Chromosome 3 with $R^{15}(+)$

TABLE 9

F_1 of four separate cultures of $2\ ci\ ey^R/ci\ ey^R\ \varphi\ \varphi \times 1\ R^{15}(+)/ci\ ey^R\ \sigma^7$.

CULTURE NUMBER	$R^{15}(+)$										$ci\ ey^R$									
	$\varphi\ \varphi$					$\sigma^7\ \sigma^7$					$\varphi\ \varphi$					$\sigma^7\ \sigma^7$				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	—	—	2	17	1	—	—	3	11	—	5	14	1	—	—	8	23	10	—	—
2	—	—	1	23	5	—	—	2	26	—	2	16	29	8	—	5	27	20	—	—
3	—	—	2	15	—	—	—	4	12	—	9	11	10	—	—	6	17	14	1	—
4	—	—	—	28	7	—	—	1	30	2	8	23	21	1	—	9	28	32	2	—
Total	—	—	5	83	13	—	—	10	79	2	2	38	77	40	1	—	28	95	76	3

insertion/normal 3, deleted 4/normal 4; (2) 3 with $R^{15}(+)$ insertion/normal 3, normal 4/normal 4; (3) normal 3/normal 3, deleted 4/normal 4; (4) normal 3/normal 3, normal 4/normal 4. Flies of genotype (3) are deficient for a section of chromosome 4. They appear phenotypically as Minutes, with extreme expression of ci but not eyeless. Obviously the deleted section includes the ci locus but not that of ey . Genotype (1) $R^{15}(+)/ci\ ey^R$ is phenotypically normal-eyed, genotype (2) insertion $R^{15}(+)/ci\ ey^R/ci\ ey^R$ appears as eyeless, as does genotype (4) $ci\ ey^R/ci\ ey^R$. Provided that genotypes (1), (2) and (4) are equally viable, a ratio of 1 $R^{15}(+)$: 2 $ci\ ey^R$ phenotypes is expected. This expectation is approached closely as shown in table 9 (the table does not include 55 Minute type flies of genotype 3). Due to the fact that in these cultures flies with $ci\ ey^R$ phenotypes include the two different genotypes (2) and (4), the mean expressivity of $ci\ ey^R$ among these flies cannot be used as a control for the expressivity of their $R^{15}(+)/ci\ ey^R$ sibs in the same sense in which it is used in the analysis of the other three $R(+)$ heterozygotes. This is borne out by the fact that the expressivity values for the $ci\ ey^R$ sibs of $R^{15}(+)/ci\ ey^R$ flies are 2.00 for females and 2.27 for males (table 10), values which lie outside the ranges of the $ci\ ey^R$ values calculated from all other groups of $R(+)$ tests (2.06–2.84 for females, 2.34–2.97 for males).

It is therefore more accurate to compare the values of expressivity of

$R^{15}(+)/ci\ ey^R$ not with those of their phenotypically $ci\ ey^R$ sibs but with the $ci\ ey^R$ values derived from the other $R(+)$ tests. The means for $R^{15}(+)/ci\ ey^R$ females vary from 2.88 to 3.20, average 3.08; and for males from 2.79 to 3.03, average 2.91 (table 10). The average expressivity for the more than 7000 $ci\ ey^R$ flies from all other tests was 2.32 for females and 2.72 for males. The $R^{15}(+)/ci\ ey^R$ heterozygotes are thus more extreme than $ci\ ey^R/ci\ ey^R$ flies, the average difference being -0.76 for females and -0.19 for males. Moreover, not only are the means for $R^{15}(+)/ci\ ey^R$ heterozygotes more extreme than the overall average for $ci\ ey^R/ci\ ey^R$, but the mean for the female heterozygotes lies also

TABLE 10

Means of expressivity of $R^{15}(+)$ heterozygotes and $ci\ ey^R$ sibs listed in table 9.

CULTURE NUMBER	$R^{15}(+)$		$ci\ ey^R$	
	♀ ♀	♂ ♂	♀ ♀	♂ ♂
1	2.95	2.79	1.80	2.05
2	3.14	2.93	1.78	2.29
3	2.88	2.75	2.03	2.26
4	3.20	3.03	2.28	2.38
Total	3.08	2.91	2.00	2.27

beyond the whole range of all means for $ci\ ey^R/ci\ ey^R$ females (range 2.06–2.84), while only one of the means for $ci\ ey^R/ci\ ey^R$ males (range 2.34–2.97) surpasses, and but slightly so, the mean for the male heterozygotes.

$R^3(+)/ci\ ey^R$

Genetic Interpretation

Twelve cultures are available for an evaluation of this constitution (table 11). The following peculiarities deserve discussion:

(1) All cultures deviate from equality of the two classes $R^3(+)$ and $ci\ ey^R$ in the direction of a deficiency of the latter class.

(2) The deficiency of $ci\ ey^R$ offspring is extreme in cultures 6–8.

(3) In addition to the flies recorded, there appeared in culture 9 one weak Minute-type male with eyeless phenotype and the most extreme expression of ci , class 4. Numerous black pupae occurred in all cultures which contained adult flies, unable to hatch, of phenotype Minute, eyeless, presumably of the same constitution as the single $M\ ey^R\ ci$ male (the venation type cannot be ascertained in unhatched adults).

(4) The distribution of $R^3(+)$ females over the phenotypic range of vein abnormalities shows in addition to the peak at class 4 an additional peak at class 2. Normally the distribution of ci shows only a single maximum.

The peculiarities (1), (3), and (4) are intelligible if it is assumed that the $R^3(+)/ci\ ey^R$ P-males form not only gametes resulting from regular segregation

but also frequently undergo nondisjunction of the normal chromosome 4 and the rod shaped chromosome consisting of the base of 4 and the translocated tip of 3 (consideration of the independent chromosome unit consisting mainly of section 95D/E-97C1, and of the corresponding deficiency will be omitted). Regular segregation leads to the balanced gametes containing all rearranged [$R^3(+)$] or all normal ($ci\ ey^R$) chromosomes and to various unbalanced gametes all of which are deficient for sections of either chromosome 2 or 3, except for a combination consisting of normal chromosomes 2 and 3 and the base 4-tip 3 fragment as a duplication. The balanced gametes $R^3(+)$ and $ci\ ey^R$ lead to

TABLE 11

F_1 of 12 separate cultures of 2 $ci\ ey^R/ci\ ey^R$ ♀ $\times$ 1 $R^3(+)/ci\ ey^R$ (?) (see text) ♂.

CULTURE NUMBER	$R^3(+)$										$ci\ ey^R$									
	♀ ♀					♂ ♂					♀ ♀					♂ ♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	—	1	3	4	12	—	1	6	7	7	—	1	6	9	—	—	—	2	5	—
2	—	—	8	2	14	—	—	4	5	12	—	—	4	10	1	—	—	1	10	—
3	—	—	1	1	5	—	—	1	5	15	—	1	1	2	—	—	—	1	11	—
4	—	1	8	7	20	—	—	4	9	17	—	—	8	8	—	—	—	4	10	—
5	—	—	4	3	9	—	—	1	9	17	—	1	4	4	—	—	1	1	9	—
6	—	—	4	4	17	—	—	1	5	12	—	—	1	—	1	—	—	—	3	1
7	—	—	4	4	18	—	—	2	14	12	—	—	1	—	—	—	—	—	7	2
8	1	—	8	5	23	—	—	3	7	21	—	—	1	8	—	—	—	—	4	—
9	—	—	—	4	14	—	—	1	12	18	—	2	15	5	—	—	—	10	10	—
10	—	—	9	1	20	—	—	—	4	18	—	—	4	14	3	—	—	2	10	4
11	—	—	3	4	26	—	—	2	18	23	—	1	7	12	7	—	2	5	8	6
12	—	1	7	3	23	—	1	7	7	9	—	2	11	10	—	—	—	2	14	—
Total	1	3	59	42	201	—	2	32	102	181	—	8	62	83	12	—	3	28	101	13

$R^3(+)/ci\ ey^R$ and $ci\ ey^R/ci\ ey^R$ offspring in equal numbers. The unbalanced gametes do not produce viable zygotes, with the possible exception of the last named type (see below). Zygotes originating from this type are deficient for most of chromosome 4 and duplicated for the tip of 3. Each one of these two aberrations separately reduces viability greatly, and the combination is probably fatal. Possibly some of the Minute ey^R black pupae may be accounted for by these unbalanced genotypes, but the rest of the inviable pupae presumably owe their origin to one of the gametes resulting from nondisjunction of chromosome 4 and the rod-shaped fragment.

The nondisjunctional gametes are of four types: rearranged set and additional 4; rearranged set deficient for base 4, tip 3; normal set minus 4; and normal set plus base of 4, tip 3. The first type leads to $R^3(+)/ci\ ey^R/ci\ ey^R$ offspring, the second to a lethal condition, the third to haplo-4 $ci\ ey^R/o$, and the last to $ci\ ey^R/ci\ ey^R$ /dupl. (base 4, tip 3). Type I, triplo-4, $R^3(+)/ci\ ey^R/ci\ ey^R$ is individually indistinguishable from the regular diplo-4 $R^3(+)/ci\ ey^R$ type. It was to be expected however, that the mean expressivity of the two genotypes would be different. Since there are two peaks in the phenotypic distribution of the $R^3(+)$ females in table 11, and since the main peak falls on the most ex-

treme class, it is concluded that this class represents mostly the regular $R^3(+)/ci\ ey^R$ flies, while the secondary peak in the $R^3(+)$ females and part of the class 3 $R^3(+)$ males are regarded as due to the presence of the $R^3(+)/ci\ ey^R/ci\ ey^R$ nondisjunctional individuals. Independent proof for this interpretation will be given below. The third type of nondisjunctional zygotes accounts for at least the greater part of the Minute ey^R pupae and the single Minute $ci\ ey^R$ male, since the phenotype of haplo-4 is Minute. The fourth type was not clearly distinguished among the offspring listed in table 11. Later tests showed it to be a $ci\ ey^R$ duplication phenotype with low viability, shortened bristles,

TABLE 12

Means of expressivity of $R^3(+)$ heterozygotes and $ci\ ey^R$ sibs listed in table 11.

CULTURE NUMBER	$R^3(+)$		$ci\ ey^R$		DIFF. BETWEEN MEANS	
	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂
1	3.35	2.95	2.50	2.71	-0.85	-0.24
2	3.25	3.38	2.80	2.91	-0.45	-0.47
3	3.57	3.67	2.25	2.92	-1.32	-0.75
4	3.28	3.43	2.50	2.71	-0.78	-0.72
5	3.31	3.59	2.33	2.73	-0.98	-0.86
6	3.52	3.61	3.00	3.25	-0.52	-0.36
7	3.54	3.36	3.00	3.22	-0.54	-0.14
8	3.32	3.58	2.89	3.00	-0.43	-0.58
9	3.78	3.55	2.14	2.50	-1.64	-1.05
10	3.37	3.82	2.95	3.13	-0.42	-0.69
11	3.70	3.49	2.93	2.86	-0.77	-0.63
12	3.41	3.00	2.35	2.88	-1.06	-0.12
Total	3.44	3.46	2.60	2.86	-0.84	-0.60

trident pattern on the thorax and broad, somewhat plexus-like veins. Cytological preparations proved the expected presence of the appropriate chromosome fragment in addition to two normal sets of chromosomes.

We have thus accounted for peculiarities (3) and (4) listed above. Since among the zygotes produced in consequence of nondisjunction only the $R^3(+)/ci\ ey^R/ci\ ey^R$ have good viability, these added to the regular $R^3(+)/ci\ ey^R$ flies increase the number of $R^3(+)$ phenotypes. Peculiarity (1), the larger number of $R^3(+)$ than of $ci\ ey^R$ phenotypes in cultures 1-5 and 9-12 is thus understandable. Finally, to account for peculiarity (2), it is assumed that the P-males of cultures 6-8 were duplication types: $R^3(+)/ci\ ey^R$ plus an extra base of 4 with the tip of 3. Regular segregation of the two base 4, tip 3 fragments in these males would lead to $R^3(+)$ type offspring with or without an extra chromosome 4 and to zygotes with normal chromosomes 2 and 3, the base 4, tip 3 duplication and either one or two chromosomes 4. The "haplo-4 plus duplication" genotype would be near-lethal, the "diplo-4 plus duplication" genotype would appear $ci\ ey^R$ and poorly viable. Its existence was verified cytologically.

Rare nondisjunction of the two base 4-tip 3 fragments would lead to a few typical diploid $ci\ ey^R/ci\ ey^R$ offspring.

Further evidence for the interpretation of the data in table 11 comes from tests of two $R^3(+)$ females of venation class 2. By progeny tests both were proved to have been triplo-4 $R^3(+)/ci\ ey^R/ci\ ey^R$. It was expected that regular segregation of the two $ci\ ey^R$ carrying chromosomes 4 would lead to $R^3(+)$ gametes containing both $R^3(+)$ and $ci\ ey^R$. Separate tests of 18 phenotypically $R^3(+)$ males derived from one of the two females confirmed this expectation since all proved to have carried three chromosomes 4. Finally, it was expected that $R^3(+)/ci\ ey^R/ci\ ey^R$ flies would produce equal proportions of $R^3(+)$ and $ci\ ey^R$ phenotypes in contrast to the deficit of $ci\ ey^R$ offspring from $R^3(+)/ci\ ey^R$ as listed in table 11. Observations of the relevant proportion in the progeny of twelve $R(+)$ -3 type males agreed with this expectation. Two of these 12 males, all of which were derived from one of the original $R^3(+)/ci\ ey^R/ci\ ey^R$ females, were identical with two of the 18 males cited above whose triplo-4 nature had been shown independently. A further analysis of $R^3(+)/ci\ ey^R/ci\ ey^R$ flies will be offered below under the heading "heterozygotes triplogenic for the ci locus."

Comparison between flies heterozygous for $R^3(+)$ and $ci\ ey^R$ and
their sibs homozygous for $ci\ ey^R$

As shown in the preceding discussion, the $R^3(+)$ heterozygotes listed in table 11 are made up of both $R^3(+)/ci\ ey^R$ and $R^3(+)/ci\ ey^R/ci\ ey^R$ individuals. In the chapter on triplogenic genotypes it will be shown that $R^3(+)/ci\ ey^R/ci\ ey^R$ have lesser degrees of vein interruption than $R^3(+)/ci\ ey^R$. Consequently, a mean value for the expressivity of all $R^3(+)$ flies will be lower than that from $R^3(+)/ci\ ey^R$ alone. Most of the $ci\ ey^R$ sibs are $ci\ ey^R/ci\ ey^R$, but a few of them may, in addition, contain the base 4-tip 3 duplication. An effect of this duplication on the mean expressivity, if it exists at all, is slight. It seems to involve a small shift toward greater expressivity since the mean values for the $ci\ ey^R$ types of cultures 6-8 are higher than those for most other cultures listed in table 11. Many of the values for the other cultures themselves are higher than the values for $ci\ ey^R/ci\ ey^R$ flies raised in simultaneous experiments involving other $R(+)$ alleles in which diplo-4 flies only were expected (*cf.* the expressivity values listed in tables 6 and 7).

The overall means of expressivity for each phenotype are listed in table 12. For the $R^3(+)$ females the means for separate cultures vary between 3.25 and 3.78, with an overall mean of 3.44. In contrast, the separate means of the $ci\ ey^R$ sibs vary between 2.14 and 3.00, with an overall mean of 2.60. In every culture the difference in mean value of expressivity between $R^3(+)$ and $ci\ ey^R$ females is negative, with an overall difference of -0.84. In other words, the position-allele heterozygotes are on the average nearly one class more extreme than the mutant homozygotes. For the males the same general picture holds. Individual means for $R^3(+)$ males vary between 2.95 and 3.82, for $ci\ ey^R$ sibs between 2.50 and 3.25. Again in every culture the mean value of expressivity for the $R^3(+)$

males is larger than for the *ci ey^R* males, the overall difference being -0.60 .

The differences for both females and males represent minimum values for the comparison of $R^3(+)/ci\ ey^R$ versus $ci\ ey^R/ci\ ey^R$ since, as pointed out in a preceding paragraph, the true value for $R^3(+)/ci\ ey^R$ heterozygotes is greater, and the true values for $ci\ ey^R/ci\ ey^R$ smaller than those listed for the $R^3(+)$ and $ci\ ey^R$ phenotypes.

HETEROZYGOTES TRIPLOGENIC FOR THE CI LOCUS

As has been discussed above, two of the $R(+)$ alleles—namely, $R^3(+)$ and $R^{15}(+)$ —can be obtained in the constitution $R(+)/ci\ ey^R/ci\ ey^R$. $R^3(+)/ci\ ey^R/ci\ ey^R$ flies are heterozygous for the $R^3(+)$ rearrangement and, in addition,

TABLE 13

F_1 of $ci\ ey^R/ci\ ey^R$ ♀ ♀ × $R^3(+)/ci\ ey^R/ci\ ey^R$ ♂ ♂.

	$R^3(+)$										$ci\ ey^R$									
	♀ ♀					♂ ♂					♀ ♀					♂ ♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Total	1	23	25	9	—	—	12	33	9	—	—	7	21	23	2	—	—	28	45	3
Means of expressivity	1.73					1.94					2.38					2.67				
Diff. between means											♀ ♀: +0.65; ♂ ♂: +0.73									

contain an extra chromosome 4. They are thus triplo-4 in constitution and have normal, not eyeless, eyes. $R^{15}(+)/ci\ ey^R/ci\ ey^R$ in contrast are diploid except for the duplicating insertion of section 101F1-102C4 of chromosome 4 into 3R. This insertion contains the $R(+)$ allele but not the *ey* locus. Consequently the flies are triploid for the *ci* locus ("triplogenic") but have *ey* eyes.

$R^3(+)/ci\ ey^R/ci\ ey^R$ triplo-4 are more nearly normal than $R^3(+)/ci\ ey^R$ diplo-4 individuals. This was deduced earlier from a consideration of types of offspring to be expected from $R^3(+)/ci\ ey^R$ heterozygotes and the bimodal distribution of the $R^3(+)$ phenotypes recorded in table 11. There is also independent proof for this statement. In crosses of $R^3(+)/ci\ ey^R/ci\ ey^R$ males to $ci\ ey^R/ci\ ey^R$ females most of the $R^3(+)$ offspring are triplo-4 and the $ci\ ey^R$ are diplo-4. Data from three such crosses are given in table 13. The mean of expressivity for $R^3(+)$ triplo-4 females is 1.73, for males 1.94. The values for $ci\ ey^R$ diplo-4 sibs serve as controls; they are 2.38 and 2.67, respectively. A comparison between the $R^3(+)$ and $ci\ ey^R$ values shows that the $R^3(+)/ci\ ey^R/ci\ ey^R$ flies are more nearly normal than the $ci\ ey^R/ci\ ey^R$ sibs, the differences being significant and positive for females and males alike—namely, +0.65 and +0.73. It is seen that triplo-4 $R^3(+)$ flies are not only more nearly normal than diplo-4 $R^3(+)$ but even more nearly normal than diplo-4 $ci\ ey^R$.

An identical result is obtained from the comparison of $R^{15}(+)/ci\ ey^R/ci\ ey^R$ versus $ci\ ey^R/ci\ ey^R$ versus $R^{15}(+)/ci\ ey^R$ phenotypes. In a preceding section it had been shown, that $R^{15}(+)/ci\ ey^R$ are more extreme than $ci\ ey^R/ci\ ey^R$ flies.

It had also been pointed out that the *ci ey^R* sibs of the $R^{15}(+)/ci\ ey^R$ individuals were a mixture of about equal numbers of two genotypes—namely, $ci\ ey^R/ci\ ey^R$ and $R^{15}(+)/ci\ ey^R/ci\ ey^R$ —and that the mean expressivity calculated jointly from the two genotypes was smaller than that of $ci\ ey^R/ci\ ey^R$ flies in general. It is concluded that this shift toward normality of the $ci\ ey^R$ phenotypes in $R^{15}(+)$ cultures is due to the triplogenic $R^{15}(+)/ci\ ey^R/ci\ ey^R$ flies, which thus are more nearly normal than $ci\ ey^R/ci\ ey^R$, which in turn are more nearly normal than $R^{15}(+)/ci\ ey^R$.

DISCUSSION

Position effects are recognized by changed action of genes in new positions. How this changed action is produced is not subject to direct investigation. It may be hoped, however, that data on the results of changed positions might constitute limits within which any acceptable interpretation of the causes of changed genic action will have to lie. The following discussion is an attempt in this direction.

Position effects may be thought of as either changes of genes themselves which in turn lead to changes in the immediate gene product or as changes of the gene product only. If the first is the case, the work of STURTEVANT (1925), DUBININ and SIDEROW (1935) and others has shown that the gene changes in altered positions, unlike those of typical mutations, are reversible with restoration of the original position. In the second case, that of an unchanged gene, but with changed product, two alternative possibilities may be envisaged. Either the quality of the product controlled by a position allele may differ from that of the normally located allele, or it may be the quantity of the product. In either case, it is clear that the site of the primary event is in or at the chromosome, since the changed effect is caused by the changed position within the chromosome. Whether or not genic action occurs by means of "plasmagenes" (cf. WRIGHT 1945) outside the nucleus, the original genic action which would be responsible for the production of such hypothetical units would still take place at the chromosomal gene locus.

In an earlier paper (STERN and HEIDENTHAL 1944) it was shown that certain position effects observed at the *ci* locus could be interpreted in either one of two basically different ways. On the one hand, it was possible to assign lower efficiency values in terms of results of gene-substrate interaction to the position alleles than the efficiency values of the alleles in their normal positions. On the other hand, it could be assumed that both the gene and the substrate for its immediate action remained qualitatively unchanged but that the quantity of the available substrate in the altered position was decreased. The result of such a situation would be a change in amount of immediate gene product. These alternative interpretations will now be applied to the new findings reported in the present paper. Two of these are:

- (1) Heterozygotes between certain position alleles $R(+)$ and the mutant allele *ci* are phenotypically more extreme than either of the two homozygotes.
- (2) Triplogenic heterozygotes of the type $R(+)/ci/ci$ are intermediate between the two diplogenic heterozygotes.

The first of these two results is nearly, if not completely unique. Genetic nomenclature has no term to designate the phenomenon of a heterozygote's being neither intermediate, nor additive, nor dominant in relation to the homozygotes. A new term "ultra-dominance" could designate the new relation were it not for the fact that the word "dominance" or any term containing this word implies the often mistaken idea of an antagonistic action between two alleles of which one, the "stronger," is dominant.

The second of the two results summarized above is equally unusual if considered in combination with the first. Since the hemizygotes of the four $R(+)$ alleles lead to normal phenotypes, result (1) may be expressed in the sentence: "Addition of a ci allele to $R(+)$ shifts the phenotype from normal to less nearly normal than ci/ci ." Correspondingly, result (2) would have to be phrased: "Addition of a second ci allele shifts the phenotype back to more nearly normal than ci/ci ."

An attempt to endow the four position alleles $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$ with changed attributes, as compared to the $+$ -allele, does not only require that these attributes are reversible with reversed position changes, but that they are of a unique nature as expressed in their interrelations with the recessive ci allele of the same locus.

In former papers, differences between different alleles at the ci locus have been interpreted in terms of different powers, c , of the alleles to combine with a cellular substrate, S , and of different efficiencies, e , of converting S into a product, P , which acts toward normal venation. In order to measure relative values for these genic attributes, it was assumed that different amounts of P are so correlated with phenotypic effect that the effect either increases with increasing P , or having reached an upper limit, remains constant. An interpretation on these premises of the results reported in this paper leads to the following considerations.

Since

$$P(R(+)/ci) < P(ci/ci) \quad (1 a)$$

it is concluded that

$$c_{R(+)} c_{R(+)} < c_{ci} e_{ci} \quad (1 b)$$

Since, however,

$$P(R(+)/R(+)) > P(ci/ci) \quad (2 a)$$

and also

$$P(R(+)/M-4) > P(ci/M-4) \quad (2 a^1)$$

it is concluded that

$$c_{R(+)} c_{R(+)} > c_{ci} e_{ci} \quad (2 b)$$

Deductions (1 b) and (2 b) are incompatible. It follows that the theoretical premises are insufficient to explain the data.

It might be suggested that the complicated facts related to the $R(+)$ alleles

could be accounted for if the relation between quantity of P and phenotype were represented by a more complex curve than one which increases to and remains at an upper limit. If we consider the two alleles, + and *ci* and, discarding the analysis into c and e attributes, assign to each a single value of activity, as for instance three for *ci* and ten for +, we would expect increasing activity in the series 1*ci*=3, 2*ci*=6, 3*ci*=9, 1+=10, +/*ci*=13, 2+=20, 3+=30. Actually the phenotypic approach to normality corresponds to the postulated values for 1*ci*, 2*ci*, 3*ci* and 1+. However, the phenotype of +/*ci* drops significantly even if only slightly below that of +, with the phenotypes of 2+ and 3+ rising again to complete normality. Values assigned to the R(+) alleles discussed in this paper and based on the phenotype of the hemizygotes would have to be between nine and ten. The phenotypes of the combinations R(+)/*ci* with activity values expected from cumulative action values of between 12 and 13 lie in reality below those of the phenotype *ci/ci* with its value of six. In other words, the relation between activity values and observed phenotypes would have to be represented by a curve rising to a maximum, then dropping deeply and again rising to an upper limit. Consideration of other alleles and combinations would add to the complexity. While such involved relations are not unknown in physiological phenomena, their *ad hoc* introduction for genic action appears not very satisfactory.

Having rejected interpretations in terms of altered c and e attributes of the position alleles and in terms of complex curves for gene activity versus phenotypic expression, we proceed now to the *assumption of reduced amount of available substrate*. If this hypothesis is made, facts reported by STERN, SCHAEFFER, and HEIDENTHAL (1946) require a specification within the framework of the hypothesis. It was shown that on the average heterozygotes of the type R(+)/*ci* are less nearly normal in phenotype than heterozygotes of the type R(*ci*)/+. Since a reduction of available substrate would on the average amount to the same quantity in R(+) and R(*ci*) rearrangements, it follows that the + and *ci* alleles of the heterozygotes do not share the substrate independently of their position. The facts become intelligible if the *normally located allele has an at least partial priority on the substrate, S, with the position allele sharing S to a secondary degree*. The hypothesis might be represented by a diagram (fig. 2): a field is delimited inside of which the normally located allele and outside of which the position allele is placed. In figures 2 a-e homozygous, hemizygous, and heterozygous combinations of the normally located allele + and *ci* are depicted in order of decreasing normality of phenotype. Figures 2 f-l shows various constitutions involving position alleles. Since different position alleles have different effects, under the hypothesis assumed to be due to individually different degrees of reduced available substrate, a special diagram would be required for each position allele. The various degrees of reduced substrate could be illustrated by varying the distance between the position allele and the region of normal location of the *ci* alleles. Since the present communication does not attempt to analyze in detail the variability of effects of different position alleles, a constant distance of the position allele

from the region of normal location has been employed in all the diagrams, except in figure 2 h.

The various constitutions involving position alleles will now be discussed. In the case of $R(ci)/+$ (fig. 2 f), the $+$ allele in its normal position obtains sufficient S to transform it by means of the allele's high c and e attributes to a product resulting in nearly normal venation, with the ci allele in its changed position being a secondary competitor. The combined action does not deviate

FIGURE 2.—Diagrams of the location of alleles in their normal position within a field of high concentration of substrate and of the location of position alleles outside of the field. For details see text.

much from that of a nonposition, heterozygote $+/ci$ (fig. 2 c). In case of $R(+)/ci$ (fig. 2 g, h), the ci allele in its normal position obtains sufficient S as does the normally located $+$ allele in the previous case but, due to the ci allele's low efficiency (e_{ci}) produces a product that results in low degrees of venation only. The $+$ allele in its abnormal position, acting in its secondary capacity, has at its disposal only the smaller amount of S not blocked by the priority of ci . In 15 out of 19 $R(+)$ alleles investigated in the heterozygote $R(+)/ci$ (fig. 2 h) the secondary contribution of the $+$ allele with its high e value added to the primary contribution of the ci allele results in a total action which is more nearly normal than homozygous ci/ci . In the four $R(+)$ alleles (fig. 2 g) which form the basis of the present study so small an amount of S is left for the position-alleles, that the combined effect of $R(+)$ and ci remains below that of ci/ci .

Similarly, in the tripogenic constitutions $R(+)/ci/ci$ (fig. 2 i) the two normally located ci alleles with their priority on S produce an effective amount of P not unlike that in a ci/ci homozygote, and the $R(+)$ position allele adds

further toward normality of venation. The result is the shift in phenotype from the extreme abnormality $R(+)/ci$ to the type of venation $R(+)/ci/ci$ which is intermediate between the two diplogetic heterozygotes.

The hypothesis developed here accounts also for the fact that hemizygotes of the $R(+)$ alleles (fig. 2 j) lead to normal phenotypes. In these cases there is no primary allele present in a normal position which would obtain a priority on S. As a result, the position allele $R(+)$ is provided with a sufficient amount of S. The same reasoning applies to the homozygote $R^{15}(+)/R^{15}(+)$ with its normal phenotype (fig. 2 k). Additional discussion is needed, however, in order to account for the ci phenotype of $R^2(+)/R^2(+)$. Such discussion might proceed on similar lines as that offered on the fact that hemizygotes for the normally located ci^w allele show normal veins, but abnormal legs and bristles, while homozygotes for ci^w possess abnormal venation, but normal legs and bristles (STERN and SCHAEFFER 1943a). These facts regarding ci^w were interpreted in terms of two different reactions competing for the same substrate, and assuming that only one of these reactions contributed toward normal venation. In the case of $R^2(+)$ it might be likewise postulated that the reduction of S available to the position allele is not due only to its unfavorable position but also to competitive removal of S at its normal position by the translocated chromosome 3R. A more detailed treatment together with additional data will be presented at a later date. Altogether, under these assumptions a position effect is regarded as dependent upon (1) the changed position of the gene whose effect is studied, (2) the specific allele of the gene concerned, and (3) the type of translocated chromosomal material which has taken the original place of the position allele.

The hypothesis of reduced S and priority of a normally located allele sheds light on a result reported earlier (STERN and HEIDENTHAL 1944): heterozygotes between $+$ - and ci -position alleles, $R(+)/R(ci)$ (fig. 2 l), are less abnormal in venation than $R(+)/ci$ heterozygotes. This was unexpected since $R(ci)/ci$ leads to a lesser degree of venation than ci/ci . It had been supposed therefore that substitution of $R(ci)$ for ci in $R(+)/ci$ would also lead to greater abnormality instead of the observed opposite effect, an approach toward normality. Under the assumption of priority on S for the typically located ci allele, but lack of priority for the $R(ci)$ allele, the observed result of $R(+)/R(ci)$ could have been predicted: lack of priority hinders the $R(ci)$ allele with its low efficiency from depriving the $R(+)$ allele of as much S as the ci allele would have done.

There is, however, one property of the $R(+)/R(ci)$ heterozygotes which does not follow from our premises. This is a relation according to which those of the five $R(ci)$ alleles tested which cause an extreme ci phenotype in $R(ci)/+$ produce the least extreme expression in $R(ci)/R(+)$. Further data are accumulating which add to the information on position effects at the ci locus. It will remain for the future to evaluate whether the sum of all data supports the hypothesis presented, how much modification of the hypothesis is needed, or whether it will have to be abandoned altogether.

The somewhat formal hypothesis of the mechanism of position effects at the *ci* locus will finally have to be translated into terms of cellular physiology. The assumptions made do not imply random distribution of the postulated substrate throughout the nucleus, but rather some kind of diffusion of S from a region of higher concentration. This region is near to the normal locus of the + or *ci* allele. The assumption of priority on S for the normally located allele with its implied blocking of S on its way to the position allele may be interpreted in terms of preferential, or exclusive distribution of S along the chromosomes from its region of high concentration. It is a fact that the majority of the striking position effects at the *ci* locus are correlated with a removal of the allele from the heterochromatic proximal region of chromosome four. Does this region provide the substrate for the cubitus alleles, and does this substrate by means of the common chromocenter reach the position alleles in their new regions within, or at the distal ends of other chromosomes? Are these assumptions compatible with our knowledge of physiological processes occurring in very small volumes and perhaps involving very small numbers of molecules?

It may be recalled that the position-allele $R^2(+)$ occupies its normal locus in an unchanged, right arm of chromosome four. The associated translocation has occurred in the left, heterochromatic arm and resulted in an attachment of most of chromosome 3R to 4L. Cases like this may, at a later occasion, serve to define more closely the factors involved in the priority hypothesis.

In order to account for position effects, the fact of somatic pairing of chromosomes in the Diptera has been used at various occasions (for example, STURTEVANT 1925; MULLER 1935; EPHRUSSI and SUTTON 1944; STERN and HEIDENTHAL 1944; GERSH and EPHRUSSI 1946). The present hypothesis, while still referring to the somatic association of heterochromatin within the chromocenter, does not require somatic pairing in heterozygotes, involving position alleles. This is an advantage, since in some cases of position effects cytological data do not support the assumption of somatic pairing in heterozygotes between position alleles and normal alleles. Thus, in $R^3(+)/ci$ no cases have been observed in salivary gland nuclei in which the $R(+)$ locus, translocated to the tip of the long rearranged 2L-3L-4R autosome, was paired with the normal chromosome four, located at the chromocenter, nor was the $R^{15}(+)$ allele, inserted in 2L, ever seen to pair with the normal chromosome four. A few early mitotic prophase in $R^3(+)/ci$ individuals likewise showed the normal chromosome four well removed from that region of the long compound autosomal arm—namely, its tip—which contains the homologous translocated section of the fourth chromosome. While the conditions in the salivary gland or oogonial nuclei may not necessarily be like those in the cells responsible for the venation effect, we regard the inference from one to the other as reasonable.

SUMMARY

Out of 19 position alleles, $R(+)$, in which a chromosomal rearrangement had taken place close to the locus of the + allele of the recessive mutant gene cubitus interruptus (*ci*) of *Drosophila melanogaster*, four $R(+)$ alleles showed

the following peculiarity: Both female and male heterozygotes $R(+)/ci$ exhibit a more extreme degree of interruption of wing veins than the homozygote ci/ci .

Hemizygotes of the constitution $R(+)/M-4$ for all four $R(+)$ alleles, designated as $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$, are normal in phenotype, with probably a slightly greater tendency for weak degree of ci venation than in $+/M-4$.

Homozygotes for $R^3(+)$ and $R^{15}(+)$ are not viable. $R^{12}(+)/R^{12}(+)$ appear normal, $R^2(+)/R^2(+)$ similar to ci/ci .

The heterozygotes $R(+)/ci$ for all four $R(+)$ alleles are more extreme than the $R(+)$ hemizygotes and the ci homozygotes. The heterozygotes between $R^2(+)$ or $R^{12}(+)$ and ci are more extreme than either of the two homozygotes.

Triplogenic heterozygotes of the types $R^3(+)/ci/ci$ and $R^{15}(+)/ci/ci$ were obtained. They are intermediate in phenotype between the two diplogenic heterozygotes.

Cytologically the rearrangements $R^2(+)$ and $R^{12}(+)$ involve a break in the right arm of chromosome 4 between the ci locus and the kinetochore. $R^3(+)$ involves a break in the left arm of chromosome 4. $R^{15}(+)$ consists of an insertion of a section of chromosome 4, right arm including the ci locus into chromosome 2.

The properties of the $R(+)$ combinations cannot be accounted for satisfactorily (a) by assigning combining powers, c , and efficiencies, e , to the position alleles different from the c and e attributes of the normally located $+$ allele, nor (b) by assuming a complex relation between varying quantities of gene product and phenotype.

A hypothesis is developed according to which the attributes of position alleles remain unchanged whereas the amount of substrate, S , available to a position allele is reduced as compared to that present in the original position. This hypothesis demands the specification that in heterozygotes the normally located allele has an at least partial priority on S , with the position allele sharing S to a secondary degree. Altogether, a position effect is regarded as dependent upon (1) the changed position of the gene whose effect is studied, (2) the specific allele of the gene concerned, and (3) the type of translocated material which has taken the original place of the position allele.

The proposed hypothesis does not require somatic pairing in heterozygotes involving position alleles.

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