AN IMMUNOGENETIC ANALYSIS OF WHITE VARIEGATED POSITION EFFECTS IN DROSOPHILA MELANOGASTER

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KATHRYN E. FUSCALDO

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By

Kathryn E. Fuscaldo

AN ABSTRACT

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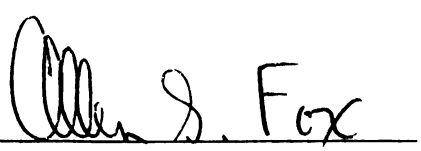
Approved 

ABSTRACT

AN IMMUNOGENETIC ANALYSIS OF WHITE

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by Kathryn E. Fuscaldo

The white variegated position effect was analyzed immunochemically to determine the effect of eu-heterochromatic rearrangements on protein structure and specificity. Stocks were used which contained the inversion, In(1) w^{m4}, a derivative, In (1) w^{m4w} and a translocation, T(1:4) w^{m5}. The mutants w, w^{e}, w^{a2} and bw cn were also tested along with Oregon-R-I. In all cases an alteration of the heterochromatic relationship to the white locus resulted in a change in the form of an antigen, designated H(w)-1.

Agar-diffusion techniques were employed to determine the antigenic specificities present in all of the stocks. The antigen H(w)-1 exhibits a higher combining power in the inverted and translocation stocks than in the wild and mutant stocks. The difference is most probably associated with a difference in the number of combining sites on the antigen along with a difference in the configuration of the antigenic site.

This alteration in the immunological properties of an antigen without a change in its specificity is associated with the disturbance of the normal euchromatic-heterochromatic balance in the cell. It is suggested that heterochromatin is involved in the final stages of

protein synthesis and more particularly in determining tertiary structure of the protein. The primary structure, that is the sequence of amino acids, is controlled by the individual, euchromatic, loci. An array of potential tertiary configurations is possible for each primary structure but the final configuration which confers absolute specificity on the protein is imposed at a later stage under the control of the heterochromatic regions.

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I. INTRODUCTION

The problem of the nature and functions of the heterochromatic portions of the chromosomes has long held the interest of geneticists. This part of the chromosomal material was at one time thought to be genetically inert (Muller, 1932). However, as more intensive studies of its properties have been undertaken, the list of functions with which it has been endowed has grown longer. Indeed, heterochromatin has been charged with so many responsibilities in the metabolism of the cell, that it takes on considerable importance in many theories of cellular behavior. It was the purpose of this investigation to determine the role of heterochromatin in protein synthesis and the determination of protein specificity.

Heterochromatin is a special type of chromatin which was first characterized cytologically. Heitz (1928) described heterochromatin on the basis of its differential staining reactions in interphase or prophase nuclei. In Drosophila melanogaster the Y chromosome and the chromocentral regions of the X chromosomes and autosomes are heteropycnotic, that is, they stain deeply while the euchromatin stains lightly or not at all. This "out of phase" property usually vanishes during metaphase. The heteropycnosis exhibited by heterochromatin has been attributed variously to differences in nucleic acid content of the heteropycnotic regions and the rest of the chromosomal complement (Levan, 1946) or to a difference in coiling (Wilson and Boothroyd, 1941 and 1944; Ris, 1945; and Coleman, 1943).

Cytochemical studies involving the use of purified nucleases and proteases have demonstrated the presence of both RNA and DNA in the euchromatic as well as the heterochromatic regions of the salivary chromosomes in Drosophila. However, there are indications that there may be more RNA in the heterochromatic areas. The chromosomes appear to be integrated fabrics of both nucleic acids and proteins (Kaufmann et al, 1951; Kaufmann, 1957).

Three types of heterochromatin have been distinguished by Heitz (1933). Alpha heterochromatin is that found in somatic interphase chromosomes. The beta heterochromatin is found in the chromocentral regions, the Y chromosome and the proximal third of the X. A third type, called intercalary heterochromatin, is indistinguishable cytologically but its existence has been inferred from the similarity of behavior with beta heterochromatin. It is presumably present as single bands or blocks distributed in the euchromatic regions of the chromosomes. Intercalary heterochromatin exhibits stickiness, high breakability when exposed to x-rays and the ability to affect euchromatic loci in a variable way. However, the difficulty of characterizing these regions cytologically casts doubt on the advisability of differentiating intercalary regions as distinct from the euchromatin.

Heterochromatin was first thought to be inert because these regions were not necessary for viability and it was thought that they had no effect on the phenotypic expression of other genes. This concept has lost ground

to theories which suppose a more active role for heterochromatin. On the basis of quantitative studies of the variation in the number of bristles in Drosophila males and females, Mather (1941, 1944) postulated a series of polygenes which control quantitative variation. This has not been substantiated since the general distribution of intercalary heterochromatin precludes any positive identification of heterochromatin with polygene activity. Goldschmidt (1949), on the basis of the podoptera effect, maintains that heterochromatin has a genetic action comparable to euchromatin but that its action is upon early differentiation. Cooper (1959) similarly believes that there is nothing peculiar about heterochromatin. Rather its differential effects are attributable to behavioral peculiarities and not to any fundamental difference in the chromosomal material.

There are some eight categories of effects which have been attributed to heterochromatin. Cooper (1959) has reviewed these and they include 1) the direct action of heterochromatin on the genic material and on gene action, 2) the stabilizing action on kinetochores and chromosone ends, on pairing at meiosis and chiasma localization. 3) Heterochromatin is active transchromosomally and in influencing variegation. 4) It has metabolic activity in mediating nucleic acid synthesis. 5) It is active in governing mitosis and 6) in development by regulating growth rates and differentiation. 7) It is suspected of having a role in sex determination and in providing the means for gene duplications that may acquire new

functions. Finally, 8) heterochromatin has been charged with being the "seat" of the unorthodox in genetic systems.

These many functions of heterochromatin may ultimately be resolved if it is true as Fox (1959) has suggested that the heterochromatic regions are concerned with the final stages of protein synthesis during which the tertiary structure of the protein is established. The evidence for this comes from a number of sources. Schultz's work (1956) on variations in nucleic acid metabolism due to the heterochromatic material can be viewed in this light. It was found that heterochromatin in the form of the Y chromosome is active in the metabolism of the nucleic acids during the formation of the egg. It changes the base constitution of RNA in the mature egg cytoplasm, but affects neither the total amount of RNA (Levenbook, Travaglini and Schultz, 1958) or the concentration of the nucleoside precursors. The behavior of the Y chromosome is equated by Schultz to all of the heterochromatin.

Schultz (1956) postulates that heterochromatin provides the basis of a feedback system in nucleic acid synthesis. If during the course of evolution, some unit which was responsible for nucleic acid synthesis were to have replicated, it would produce a region composed of homologous genes having very little apparent specific effect because of their duplication. Cooper's (1956) suggestion that the differential heterochromatic effects are due to disturbances in the euchromatic-heterochromatic balance are pertinent here.

Fox's suggestion that heterochromatin is concerned with tertiary protein structure gains credence from considerations of this kind. If particular euchromatic genes are responsible for determining the sequence of amino acids in a protein, that is, for specifying primary structure, a possible array of tertiary structures would be provided. Heterochromatin would then specify the particular tertiary configuration assumed by the protein. That genes determine primary structure has been demonstrated by Ingram in the case of the amino acid sequence in the hemoglobins (1956). The evidence that heterochromatin is involved in determining tertiary structure is more circumstantial, but none-the-less compelling. Fox (1958) has shown that the Y chromosome has a maternal effect on protein structure. An antigen, designated Y-1, has a complete and an incomplete form. The complete form will induce antibody formation, will unite with the antibody, and will form a visible precipitate. The incomplete antigen on the other hand will induce antibody formation, will unite with the antibody, but will not form a precipitate. The presence or absence of the Y chromosome in the oöcyte of a female determines the form of the antigen in her progeny, but the specificity of the antigen remains the same.

One of the striking effects of heterochromatin is the production of variegated position effects (Lewis 1950). The term position effect was proposed by Sturtevant (1928) to describe changes in the phenotypic effects of genes occasioned by changes in their position in the genome. Chromosome rearrangements disturbing the relationships of euchromatin

and heterochromatin frequently result in variegated position effects. More specifically, when a euchromatic locus is brought into the proximity of heterochromatin by such a rearrangement, a mosaic phenotype is induced in which patches of mutant tissue are interspersed with an otherwise wild background. The first such case discovered (w^{m1}, Muller, 1930) involved the white locus. In this case, the left end of the X chromosome was broken to the right of the white locus and was transferred to the proximal heterochromatin of the X. The phenotypic effect was to produce patches of white ommatidia in the otherwise wild type eyes.

Mosaicism of this type is extremely variable. In the first place, the mutant phenotype is expressed in some cell lineages but not in others. It is affected to a marked degree by environmental conditions such as temperature and overcrowding of culture bottles. A threshold effect seems to be involved, such that irreversible differentiation either in the normal or mutant direction takes place in different cell lineages.

Dubinin and Siderov (1935) and Panshin (1935) were able to demonstrate that a gene showing such a variegated or "V-type" position effect when close to a break involved in a euchromatic-heterochromatic rearrangement reverts to normal in its phenotypic affects when restored to its normal position by crossing-over. The decisive factor in the determination of variegation in the w^{m4} rearrangement was shown by Schultz (1943) to be located at the junction between the white locus and the heterochromatin. This was demonstrated by crossing-over between the white-variegated (w^{m4})

and other not quite identical inversions. Judd (1959) has also demonstrated this with other white-variegated rearrangements. It thus appears that the influence exerted by heterochromatin on euchromatic loci is the primary contribution to V-type position effects.

The ability of heterochromatin to modify the action of a locus extends not only to that gene nearest the inserted heterochromatin, but also to others further removed from the rearrangement. This is the so-called spreading effect (Lewis, 1950). The spreading effect necessitates a linearly differentiated chromosome with the units (genes) having specific developmental effects.

A number of mechanisms have been suggested as being responsible for the production of V-type position effects by heterochromatin (Hannah, 1951; Lewis, 1950). These include the positions taken by 1) Muller (1935), Offerman (1935), and Stern (1949) that a change in interaction of gene products or competition between genes for a common substrate is the cause: 2) Ephrussi and Sutton (1944), that reversible modification of the genes or their structure due to abnormal pairing relationships is responsible: 3) Goldschmidt (1946), that the production of the phenotype of a mutant locus by a break occurring in the neighborhood of the normal locus results from the disruption of a field of action extending over a considerable segment of the chromosome; and 4) Schultz (1941) and Prokofieva-Belgovskaya (1937), that the heterochromatin induces a change in the euchromatic loci newly brought into its proximity such that they assume some of the

properties of heterochromatin, i.e. are "heterochromatized".

The system that was chosen in the present work for an elucidation of the role of heterochromatin is one that involves a disturbance of the euchromatic-heterochromatic balance leading to a variegated expression of the white phenotype. The white segment itself is composed of four pseudoallelic loci. The most distal locus is occupied by one of a multiple allelic series including mutants, of which white-buff (w^{bf}) is typical. The locus to the right of this one is represented by the mutant white-apricot (w^{a}) among others. The next most proximal locus contains the mutants white and white-eosin (w^{e}), as well as five others. The last site is occupied by the mutant white-spotted (w^{sp}). The white region therefore is a pseudoallelic segment involving about twenty-one alleles distributed among four separable, although spatially related, loci. The whole segment is located in the region of bands 3C1 and 3C2 of the X chromosome in the Drosophila salivary chromosome maps. (Warren, personal communication).

Immunochemical analyses of the stocks containing alterations leading to variegated phenotypes at the white region were undertaken to determine the effects of such heterochromatic disturbances on protein specificity.

II. MATERIALS AND METHODS

The stocks of Drosophila melanogaster used in this study were provided by Dr. Jack Schultz.

A. DESCRIPTION OF STOCKS

1. Oregon-R-I. An isogenic, wild type stock maintained by A. S. Fox. Originally isogenized by J, Schultz, and maintained through 230 generations of brother-sister matings at the start of this work.

2. Oregon-R-I (S). A subline of Oregon-R-I maintained in the laboratory of J. Schultz. The two lines of Oregon-R-I have been maintained separately approximately since generation 50 of brother-sister mating.

3. gll. In(1)w^{m4}. An inversion resulting from two breaks and subsequent rearrangement of the X chromosome (Bridges and Brehme, 1944). The proximal break occurred in the chromocentric heterochromatin to the right of bobbed (bb) in section 20 of the salivary chromosome map of the first (X) chromosome. The distal euchromatic break occurred between bands 3C1 and 3C2 to the right of white. The result of this inversion was to bring the heterochromatin normally located in the chromocentric region into the proximity of the white pseudoallelic region. The phenotypic expression of the rearrangement is in a variable mosaic pattern of

pigment deposition in the ommatidia of the insect eye. Although in this case there is probably no change in the gene itself (Schultz, 1943) some of the ommatidia exhibit the mutant white phenotype. This stock was rendered co-isogenic with Oregon-R-I by Schultz, but since then it has been carried as a separate line without further outbreeding to the wild stock. When received from Schultz, the stock carried extra, free Y chromosomes which were expressed in terms of suppression of variegation. By selecting for extreme variegation, these extra Y's were eliminated. The success of this removal was confirmed by cytological examination of larval cerebral ganglia.

4. g12. In (1)w^{m4w}. (Schultz, 1943). A derivative of In(1)w^{m4}, exhibiting more extreme variegation. No change in the rearrangement is detectible cytologically, and the change probably occurred in the white region itself.

5. g13. In (1)w^{m4w}; Y^{su-V}. A stock possessing the same rearrangement as in g12, but with an altered Y chromosome which suppresses variegation. The stocks originally contained extra Y's, but these were removed by selection of the most highly variegated phenotypes. Removal of the extra Y's was confirmed by cytological examination.

6. g14. In(1)w^{m4w}; Df(Y)Y^{-bb}. Possesses the same X chromosome as g12, but with a Y chromosome deficient for the bobbed locus. Cytological examination revealed no extra Y's.

7. h8. T(1:4)w^{m5}. A stock which is homozygous for a reciprocal translocation between the X and fourth chromosome. (Bridges and Brehme, 1944). Of the two breaks involved, one is in the distal tip of the X between bands 3C2 and 3C3, to the right of white and to the left of facet, and the second in the fourth chromosome between bands 101F1 and 101F2 between bent (bt) and cubitus interruptus (ci). A reciprocal translocation of the products of the breaks put the tip of the X carrying the white region onto the chromocentric portion of the fourth, carrying the cubitus interruptus locus and almost all of the fourth onto the end of the X chromosome. The result of the alteration was to place the normally euchromatic white region adjacent to a heterochromatic area with the consequence of producing a variegated phenotype. The rearrangement also results in a position effect on cubitus interruptus. Both the white region and the cubitus interruptus locus, however, are probably unmodified.

8. w^{a2}. white apricot 2. One of a pseudoallelic series of mutants in the white region. Localized at the second locus from the distal end of the four pseudoallelic loci in the white segment (Green, 1959). Eye color orange pink, darker in males.

9. w^{e}. white eosin. One of a pseudoallelic series of mutants in the white region. Localized to the right of w^{a2} (Green, 1959). Eye color yellowish-pink, male lighter.

10. w. white. One of a pseudoallelic series of mutants for which the white region is named. Occupies the same locus as w^e. Eye color nearly snow white.

11. bw cn. brown-cinnabar. A double mutant stock, homozygous for brown (bw, 2-104.5) and cinnabar (cn, 2-57.5). Eye color white.

B. CYTOLOGY

Larvae for cytological examination were raised on standard medium at 18°C. Third instar larvae were dissected in aceto-orcein under cover slips. Permanent preparations were made by immersing the squashes in tertiary butyl alcohol and ethyl alcohol (9:1) overnight. Coverslips with squashes were then mounted in clarite on clean microscope slides for examination under oil with a Zeiss WL research microscope equipped with apochromatic objectives.

Twenty larvae were used from each stock employed in the investigation. The larvae were selected at random from at least five different cultures of each stock. Salivary chromosome preparations were examined to confirm the position of the breaks in the inversions and the translocation. Mitotic figures of cerebral ganglion cells were examined to confirm the absence of extra Y chromosomes. Photographic record was made of selected figures.

C. IMMUNOCHEMICAL TECHNIQUES

The techniques used for immunochemical analysis have been adopted from those described by Fox (1958), involving application of the agar-diffusion method described by Ouchterlony.

1. Collection of Flies for Antigen Preparation.

The stocks were mass cultured in half-pint milk bottles on standard cornmeal, molasses, agar medium and maintained at 25 C. Flies were collected from those bottles which showed no visible signs of contamination. The flies were starved for eight to ten hours. They were then frozen rapidly, lyophilized, and stored in the deep-freeze.

2. Preparation of Antigen for Immunization.

Whole, lyophilized flies were homogenized with an all glass Potter-Elvejhem homogenizer in cold 0.85% NaCl, buffered at pH 7.4 with 0.005 M phosphate, to make a 2% (w/v) homogenate. The entire homogenate was used to immunize rabbits according to the following schedule.

3. Preparation of Normal Sera and Antisera.

Normal serum was collected from each rabbit by bleeding from the ear. Merthiolate, 1:5000, was added to prevent bacterial growth and the sera were frozen and stored in the deep-freeze. Each rabbit was then immunized according to the following schedule.

Day	Antigen Dose
1	2.0 ml., interperitoneal
3	4.0 ml., interperitoneal
5	8.0 ml., interperitoneal
12	8.0 ml., interperitoneal
19	8.0 ml., interperitoneal
33	8.0 ml., interperitoneal

The rabbits were bled from the ear on day 26 in order to test for antibody titer. On day 40, they were exsanguinated by cardiac puncture. The antisera were separated, merthiolate added 1:5000, and stored in the deep-freeze.

4. Preparation of Agar Plates.

2% (w/v) agar was prepared by solution in buffered saline (0.85% NaCl buffered at pH 7.4 with 0.005 M phosphate, plus 0.01% merthiolate), filtration through one thickness of Whatman No. 1 filter paper, and sterilization in an autoclave. 30.0 ml quantities were poured aseptically into sterile, 90 mm. petri dishes and cooled slowly. After overnight storage at 5° C, wells were cut in the agar by the use of a template and sterile metal cutting tubes. Each well was sealed at the bottom with one drop of melted agar.

5. Ouchterlony Tests.

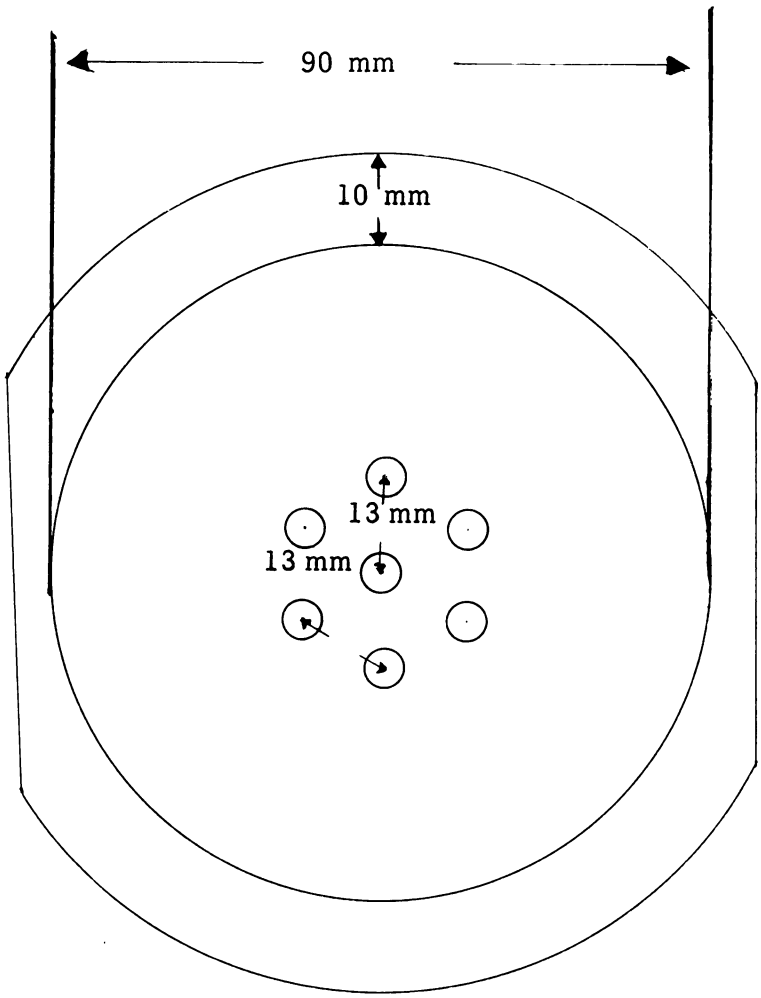
The geometrical arrangement of wells used in the Ouchterlony tests is given in Figure 1. Each well holds 0.05 ml of test solution. Antigen solutions were placed in the outer wells and undiluted antiserum in the central well. The wells were all filled simultaneously, five doses

being sufficient for development of definitive patterns of precipitate lines.

6. Bjorklund Inhibition Tests.

Antisera were inhibited by the method of Bjorkland, as adapted by Fox (1958). Inhibiting antigens were prepared from the supernatant fluids described above as follows: 1) dialysis against distilled water to remove salts; 2) lyophilization; 3) restoration to one tenth the original volume in 0.85% NaCl buffered at pH 7.4 with 0.005 M phosphate. Merthiolate was added to a concentration of 0.01%. For the purpose of inhibition, a 0.05 ml dose of the chosen inhibiting antigen was added to the center well on each of a specified number of days. The plates were allowed to stand for 24 hours without further additions, and then the Ouchterlony tests were performed as usual.

FIGURE 1. Template for Ouchterlony Plates.



The template is cut from plastic. The outer diameter is 120 mm allowing for a 10 mm beveled edge to seat the plastic disc on the petri dish which has a diameter of 90 mm.

III. RESULTS

Table 1 lists the antisera used in the analysis. At least one antiserum against each of the genotypes except w^e, w^{a2} and bw cn are included in the list. Additional antisera were tested but yielded no specific information.

Ouchterlony tests with uninhibited antisera yielded plates exhibiting from four to seven precipitate lines (Plates I, II, IV), depending on the particular serum tested. Most of the lines were of no special interest since they were continuous between antigen wells, and consequently common to all of the stocks. These antigens are presumably products of those portions of the genotype which are the same in all of the stocks. One antigen, however, exhibited a distribution among the stocks which was correlated with genetic differences. This antigen will be referred to as H(w)-1, since it will be shown that the differences which it exhibits in the various stocks are attributable to effects of heterochromatin on the white pseudoallelic segment.

H(w)-1 is an antigen, present in all of the inversion stocks, (gl1,gl2,gl3,gl4), capable of inducing the formation of antibodies and able to unite with these antibodies to form a precipitate line in the agar (Plate I). In each case, however, the H(w)-1 line is either shifted toward the antigen well with a concomitant decrease in density (Plate Ia, b), or is absent (Plate Ic, d), opposite the wells containing the wild type antigens (Oregon R-I and Oregon R-I (S)). The shift in position is

characteristic of the behavior of these antigens with S-338, S-336, and S-314 (Table 2). The line is absent when S-337 is used (Table 2).

Inhibition is of two types with respect to H(w)-1. Inhibiting antigens prepared from the inversion stocks are completely effective (Plate III b) preventing the formation of all lines when five inhibiting doses are used. Wild type inhibition, on the other hand differs in two ways. First, at a dose which provides complete inhibition when the inversion antigens are used (5 doses), the H(w)-1 line remains after inhibition with the wild type antigens. (Plates III a,e). Second, inhibition at this dosage is partial, i.e. the H(w)-1 line is formed with the inversion antigens, but not with the Oregon-R-I or Oregon-R-I (S) antigens. This is true regardless of whether the line was evident or not opposite the wild type antigens on uninhibited plates of a particular antiserum. Complete inhibition of all lines was effected however, with the wild type inhibiting antigens when 10 inhibiting doses were used. On the basis of this behavior which is summarized in Tables 2 and 3, the antigen H(w)-1 is said to be normal in the inversion stocks, but modified in the Oregon-R stocks. Tests with the stock h8, which is characterized by a translocation involving a disturbance of the eu-heterochromatic relationship at the white region, were performed to determine whether the behavior of H(w)-1 was peculiar to the inversions or whether a more generalized phenomenom attributable to heterochromatic disturbances was responsible. Plate IV demonstrates that in the translocation stock, h8, the antigen H(w)-1 is normal as in the inversion stocks. (Summary in Tables 2 and 3). In order

to investigate the role of the white region itself with regard to the form of the antigen, tests were performed with the white mutants. The behavior, in Ouchterlony tests, of H(w)-1 antigen in white (Plates II, IV) and white-eosin (Plate IV a,b) parallels that of the antigen in Oregon-R stocks (Tables 2 and 3). However, white and white-eosin effect complete inhibition at a lower dose than the Oregon-R-I or Oregon-R-I (S) antigens (Plate III c; Table 3). White-apricot on the other hand, behaves like the inversions (Plate IV a,b); Table 2) in that the form of the antigen is normal. White-apricot 2 is completely effective as an inhibiting antigen at the lower dose (Plate III d; Table 3).

Finally, the double mutant brown, cinnabar, giving a white phenotype, was tested. Here again the form of the antigen was like that of wild, white and white-eosin (Table 2). Brown cinnabar was also completely effective as an inhibiting antigen at the lower dose (Table 3).

The lack of antibodies to H(w)-1 in the Oregon-R-I and Oregon-R-I (S) (Tables 2 and 3) is not indicative of the inability of the antigen to induce antibody formation in these stocks. A more extensive analysis of other sera against these stocks may reveal the presence of such antibodies. The tests performed thus far are too limited to rule out such an eventuality.

The form of H(w)-1 in all of the genotypes is summarized in Table 4.

TABLE 1. List of Antisera

Antiserum	Genotypes providing immunizing antigens
S-314	g14-In(1) w^{m4w}; Df(Y) Y^{-bb}
S-336	g13-In(1) w^{m4w}; Y^{su-V}
S-337	g12-In(1) w^{m4w}
S-337a	g12-In(1) w^{m4w}
S-338	g11-In(1) w^{m4}
S-339	h8- T(1:4) w^{m5}
S-340	Oregon-R-I
S-341	Oregon-R-I (S)
S-342	white
S-345	brown, cinnabar

TABLE 2. Characteristics of H(w)-1 Precipitate line formed by antigens from different stocks with uninhibited antisera.

Antiserum	Characteristics of H(w)-1 line		
	Normal	Displaced	Absent
S-314	gl1, gl2	Oregon-R-I	
	gl3, gl4	Oregon-R-I-(S)	
	h8, w^{a2}	w, w^{e}	
S-336	gl1, gl2	Oregon-R-I	
	gl3, gl4	Oregon-R-I (S)	
	h8, w^{a2}	w, w^{e}	
S-337	gl1, gl2		Oregon-R-I
	gl3, gl4		Oregon-R-I (S)
	h8, w^{a2}		w, w^{e}
S-338	gl1, gl2	Oregon-R-I	
	gl3, gl4	Oregon-R-I (S)	
	h8, w^{a2}	w, w^{e}	
S-339	gl1, gl2	Oregon-R-I	
	gl3, gl4	Oregon-R-I (S)	
	h8, w^{a2}	w, w^{e}	
S342	gl1, gl2	Oregon-R-I	
	gl3, gl4	Oregon-R-I (S)	
	h8, w^{a2}	w, w^{e}	

TABLE 3. Behavior of H(w)-1 line following inhibition of antisera.

Antiserum	Inhibiting Antigen*	Presence or absence of H(w)-1 line when tested with										
		Ore-R-I	Ore-R-I (S)	g11	g12	g13	g14	h8	w	w^e	w^{a2}	brown
S-314	Oregon-R-I	-	-	+	+	+	+	N	-	N	N	N
(Anti-g14)	Oregon-R-I (S)	-	-	+	+	+	+	N	-	N	N	N
	g11	-	-	-	-	-	-	-	-	N	N	N
	g12	-	-	-	-	-	-	N	-	N	N	N
	g13	-	-	-	-	-	-	N	-	N	N	N
	g14	-	-	-	-	-	-	N	-	N	N	N
	w	-	-	-	-	-	-	N	-	N	N	N
S-336	Oregon-R-I	-	-	+	+	+	+	+	-	N	N	N
(Anti-g13)	Oregon-R-I (S)	-	-	+	+	+	+	+	-	N	N	N
	g11	-	-	-	-	-	-	-	-	N	N	N
	g12	-	-	-	-	-	-	-	-	N	N	N
	g13	-	-	-	-	-	-	-	-	N	N	N
	g14	-	-	-	-	-	-	-	-	N	N	N
	w	-	-	-	-	-	-	-	-	N	N	N

TABLE 3 (cont'd)

Antiserum	Inhibiting Antigen*	Presence or absence of H(w)-1 line when tested with										
		Ore-R-I	Ore-R-I (S)	gl1	gl2	gl3	gl4	h8	w	w^e	w^{a2}	brown
S-337	Oregon-R-I	-	-	+	+	+	+	N	-	N	N	N
(Anti-gl2)	Oregon-R-I (S)	-	-	+	+	+	+	N	-	N	N	N
	gl1	-	-	-	-	-	-	-	-	N	N	N
	gl2	-	-	-	-	-	-	-	-	N	N	N
	gl3	-	-	-	-	-	-	-	-	N	N	N
	gl4	-	-	-	-	-	-	-	-	N	N	N
	w	-	-	-	-	-	-	-	-	N	N	N
	w^e	-	-	-	-	-	-	-	-	-	N	N
	w^{a2}	-	-	-	-	-	-	-	-	N	-	N
	bw cn	-	N	N	-	N	N	N	-	-	-	-
S-338	Oregon-R-1	-	-	+	+	+	+	N	N	N	N	N
(Anti-gl1)	Oregon-R-I (S)	-	-	+	+	+	+	N	N	N	N	N
	gl1	-	-	-	-	-	-	N	-	N	N	N
	gl2	-	-	-	-	-	-	N	-	N	N	N
	gl3	-	-	-	-	-	-	N	-	N	N	N
	gl4	-	-	-	-	-	-	N	-	N	N	N
	w	-	-	-	-	-	-	N	-	N	N	N

TABLE 3 (cont'd)

Antiserum	Inhibiting Antigen*	Presence or absence of H(w)-1 line when tested with										
		Ore-R-I	Ore-R-I (S)	g11	g12	g13	g14	h8	w	w^e	w^{a2}	brown
S-339	Oregon-R-I	-	-	-	-	-	-	-	N	N	N	N
(Anti-h8)	Oregon-R-I (S)	-	-	-	-	-	-	-	N	N	N	N
	g11	-	-	-	-	-	-	-	-	N	N	N
	w	-	-	-	-	-	-	-	N	N	N	N
S-340	Oregon-R-I	-	-	-	-	-	-	N	N	N	N	N
(Anti-One R-I)	Oregon-R-I (S)	-	-	-	-	-	-	N	N	N	N	N
	g11	-	-	-	-	-	-	N	N	N	N	N
	g12	-	-	-	-	-	-	N	N	N	N	N
	g14	-	-	-	-	-	-	N	N	N	N	N
S-341	Oregon-R-I	-	-	-	-	-	-	N	N	N	N	N
(Anti-One R-I (S))	Oregon-R-I (S)	-	-	-	-	-	-	N	N	N	N	N
	g11	-	-	-	-	-	-	N	N	N	N	N
S-342	Oregon-R-I	-	-	-	-	-	-	N	-	N	N	N
(Anti-White)	Oregon-R-I (S)	-	-	-	-	-	-	N	-	N	N	N
	g11	-	-	-	-	-	-	N	-	N	N	N
	w	-	-	-	-	-	-	N	-	N	N	N

TABLE 3 (cont'd)

Antiserum	Inhibiting Antigen*	Presence or absence of H(w)-line when tested with										
		Ore-R-I	Ore-R-I (S)	g11	g12	g13	g14	h8	w	w^e	w^{a2}	brown
S-345 (Anti-bw cn)	Oregon-R-I	-	-	-	N	-	N	-	-	N	N	-
	Oregon-R-I (S)	-	-	-	N	-	N	-	-	N	N	-
	w	-	-	-	N	-	N	-	-	N	N	-
	bw cn	-	-	-	N	-	N	-	-	N	N	-

*Inhibition performed with 5 doses of inhibiting antigen.

+, H(w)-1, line present.

-, H(w)-, line absent.

N - No test performed.

TABLE 4. Form of H(w)-1 in Different Genotypes.

Genotype	Form of Antigen
g11, In(1) w^{m4}	normal
g12, In(1) w^{m4w}	normal
g13, In(1) w^{m4w}	normal
g14, In(1) w^{m4w}	normal
h8, T(1:4) w^{m5}	normal
w white	modified
w^{e}, white-eosin	modified
w^{a2}, white-apricot 2	normal
bw cn, brown, cinnabar	modified
wild, Oregon-R-I	modified
or	
Oregon-R-I (S)	modified

PLATE 1.

a. Antiserum: S-338

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 14
3. 13
4. I
5. S
6. 12

b. Antiserum: S-314

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 14
3. 13
4. I
5. S
6. 12

c. Antiserum: S-337

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 14
3. 13
4. I
5. S
6. 12

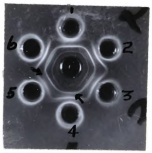
d. Antiserum: S-337

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 12
3. 13
4. I
5. S
6. 14

Arrows indicate H(w)-1 line.

PLATE 1.



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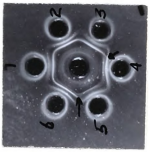

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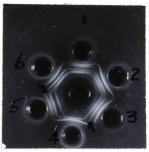

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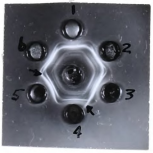


d.

PLATE 2.

a. Antiserum S-338

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 12
3. S
4. W
5. 13
6. 14

b. Antiserum S-336

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 12
3. S
4. W
5. 13
6. 14

c. Antiserum S-337

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 12
3. S
4. W
5. 13
6. 14

d. Antiserum S-314

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 12
3. S
4. W
5. 13
6. 14

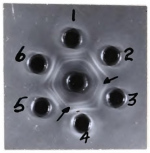
e. Antiserum S-342

Inhibiting Antigen: None

Antigen wells: 1. 11
2. 12
3. S
4. W
5. 13
6. 14

PLATE 2.

a.



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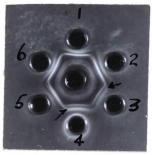

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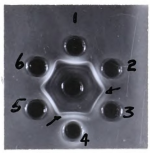

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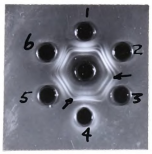

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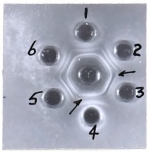


PLATE 3.

a. Antiserum S-337

Inhibiting Antigen: Oregon-R-I (S), 5 doses

Antigen wells: 1. 11
2. 12
3. 13
4. I
5. S
6. 14

b. Antiserum S-337

Inhibiting Antigen: w^e, 5 doses

Antigen wells: 1. 12
2. w
3. w^e
4. I
5. bw cn
6. w^{a2}

c. Antiserum S-337

Inhibiting Antigen: gl2

Antigen wells: 1. 11
2. 12
3. 13
4. I
5. S
6. 14

d. Antiserum S-337

Inhibiting Antigen: w^{a2}, 5 doses

Antigen wells: 1. 12
2. w
3. w^e
4. I
5. bw cn
6. w^{a2}

e. Antiserum S-337a

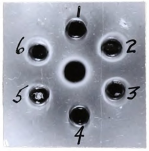
Inhibiting Antigen: Oregon-R-I, 5 doses

Antigen wells: 1. 11
2. 12
3. 13
4. w
5. I
6. 14

PLATE 3.

a.

c.



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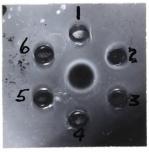


d.

e.

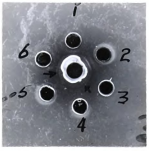


PLATE 4.

a. Antiserum: S-314

Inhibiting Antigen: None

Antigen wells:
1. 14
2. h8
3. w
4. w^e
5. w^{a2}
6. S

b. Antiserum: S-339

Inhibiting Antigen: None

Antigen wells:
1. 11
2. 12
3. 13
4. h8
5. I
6. 14

c. Antiserum: S-337

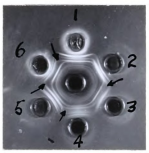
Inhibiting Antigen: None

Antigen wells:
1. 11
2. 12
3. 13
4. h8
5. I
6. w

Arrows indicate H(w)-1 line.

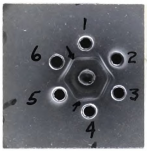
PLATE 4.

a.



b.

c.



IV. DISCUSSION

The results of the immunochemical analysis of the white variegated position effect discloses several interesting points which are pertinent to genetical theory and the mechanism of protein synthesis.

Two forms of an antigen, designed H(w)-1 were found. One form, associated with the heterochromatic region, behaves normally in that it induces the formation of antibody, combines with the antibody and precipitates it. Complete inhibition of all antisera is affected by the "normal" form of the antigen. The second form of H(w)-1, found in the stocks which have no heterochromatic alterations (w^{a2} excepted), is said to be the modified form for purely arbitrary reasons based primarily on its inhibitory activity. The modified form also induces the formation of the antibody, combines with it but less effectively than the normal H(w)-1 and precipitates it.

The displacement of the modified line can be explained in any one of three ways. A lower concentration of the H(w)-1 antigen in the wild and mutant stocks would pull the precipitate line toward the antigen well since the antigen-antibody equivalence point would be shifted. The second possibility that would account for the differential behavior of the H(w)-1 antigen would be in a reduction in the number of combining sites possessed by the modified form of the antigen. An antigen with fewer combining sites would behave as if there were less antigen present. The third alternative would involve reduction in the number of combining sites per molecule with a concomitant change in the configuration of the

antigen molecule which would reduce its combining power.

The first possibility, that of concentration differences is ruled out on the basis of the inhibition data. If the differences between the two antigenic responses were attributable to concentration, then one would not expect to achieve only partial inhibition with the modified form of the antigen. The fact that the modified H(w)-1 completely inhibits the reaction of all antisera with the wild and mutant stocks while not inhibiting the H(w)-1 line against the inversions and the translocation, precludes the possibility of attributing the observed variations in behavior to concentration differences.

The second alternative is similarly not completely satisfactory in explaining the discrepancies. If the alteration in the immunological behavior of the antigen were due merely to a reduction in the number of combining sites, then the addition of wild type inhibiting antigen in excess of five doses would not be expected to effect complete inhibition. In the region short of antigen excess, a line should appear with both the normal and modified antigens if the differences between the two were due simply to a reduction in the number of combining sites in the latter.

In view of these considerations, it seems most likely that the alteration in the form of the antigen is due both to a reduction in the number of combining sites per molecule of antigen and to a change in the configuration of the molecule affecting its combining power. The normal form of the antigen, having a larger number of combining sites and a more effective configuration for combining with the antibody, could displace

the modified form. Higher doses of the wild type inhibiting antigen would prevent such displacement. Under competitive situations, then, the normal form of the antigen is more effective in antibody union than the modified H(w)-1 antigen.

Under this system, the mutants white, white-eosin and brown-cinnabar have the modified antigen but in a higher concentration enabling them to compete with the normal antigen at a lower dosage. However, the possibility cannot be ignored that the modification in number of combining sites or in configuration is not as severe in these mutants as it is in the wild stocks.

The antigen H(w)-1 has no direct association with eye color, per se. The heterochromatic regions seem to be directly responsible for the difference between normal and modified H(w)-1, since an alteration of the white segment in In(1)w^{m4w} does not affect the form of the antigen. Any heterochromatic disturbance, whether by euchromatic-heterochromatic rearrangements within the same chromosome or by translocation of a euchromatic region of one chromosome to a heterochromatic portion of another as in T(1:4) w^{m5}, has the same result on the form of the antigen. The white segment is also involved in the production of the antigen since an alteration, w^{a2} located to the left of white behaves like the heterochromatic rearrangements. If the protein is considered to be a product of the entire pseudoallelic segment, then the heterochromatin modifies that protein in a way which parallels the effect of certain changes in the white segment itself.

The situation with respect to H(w)-1 bears a relationship to that which exists in the case of Fox's Y-1 antigen (Fox, 1959). In the latter instance the form of the Y-1 protein is modified so that it behaves as an incomplete antigen when a Y chromosome is present in the oöcyte of the mother. The heterochromatic Y, then, has an affect in the cytoplasm of the oocyte to modify the structure of an antigen in the progeny of the mother containing the Y. The presence or absence of the Y in the individual has no affect on the form of the antigen. The analogy to the H(w)-1 antigen is that both cases involve a disturbance of the heterochromatic balance in the genome. In both cases, such a disturbance affects protein structure by altering the form of an antigen which is presumably a product of one or more euchromatic loci.

It seems reasonable to assume that the heterochromatin is active during the terminal stages of protein synthesis. The euchromatic loci and heterochromatin exist in definite relationship to each other. Destruction of the balance between the two chromosomal elements results in disturbances in the metabolic activities of the cell. The disturbance is of a variable nature with respect to heterochromatic effects. It seems that a threshold exists which must be overcome before the effect is expressed phenotypically. In variegation, the threshold is surpassed in some cell lineages resulting in mutant patches on a wild background. Once the threshold has been exceeded, the outcome is irreversibly altered.

V. SUMMARY

1. Immunogenetic analysis of the white variegated position effects, w^{m4} and w^{m5} in Drosophila melanogaster revealed the presence of an antigenic component, designated H(w)-1, which displayed different immunological properties from those exhibited by the non-inverted stocks.

2. The form of the antigen in In(1)w^{m4}, In(1)w^{m4w}, In(1)w^{m4w}; Y^{su-V}, In(1)w^{m4w}; Df(Y)Y^{-bb}, T(1:4)w^{m5} and white-apricot-2 has been termed "normal" in that it displays maximum effectiveness in combining with and precipitating the antibody.

3. The form of the antigen in Oregon-R-I, Oregon-R-I(S), white, white-eosin and brown cinnabar has been designated as "modified" because of its reduced ability to combine with and precipitate the antibody.

4. The change in the form of the antigen is attributed to the activity of the heterochromatin.

5. An analogy is drawn between the results obtained from this analysis of the euchromatic-heterochromatic rearrangements on the form of the antigen H(w)-1 and those obtained by Fox in his study of a system involving alterations in an antigen, Y-1, brought about by an additional Y chromosome in the oocyte of the mother.

6. It is suggested on the basis of these and other studies that heterochromatin is involved in protein synthesis. Its most probable role is in the final stages of protein synthesis, those involving the tertiary structure of the protein.

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