THE GENETIC BASIS OF A NUTRITIONAL REQUIREMENT IN DROSOPHILA¹

TAYLOR HINTON

Department of Zoology, University of California at Los Angeles

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WITH the accomplishment of a technique for raising *Drosophila* under aseptic conditions on a synthetic medium (SCHULTZ, ST. LAWRENCE, and NEWMAYER 1946; HINTON, NOYES, and ELLIS 1951) it was possible to detect mutations that affect the nutrition of the organism. Studies of genetic changes affecting metabolic pathways have been restricted, for the most part, to microorganisms. The first change in nutritional requirement in *Drosophila* was reported in 1950 (HINTON and ELLIS) and described in detail in 1951 (HINTON, ELLIS, and NOYES). The nature of the change was an inability of the organism to grow and develop in the absence of the adenine component of nucleic acid in the diet; whereas a wild-type strain (Oregon-R) of *Drosophila* did not require nucleic acid or any of its derivatives. The present report presents data concerning the genetic nature of this nutritional requirement.

The requirement was present in a strain (*In(2LR)40d/Cy*) which contains an interbrachial inversion in the second chromosome having one break in heterochromatin. The rearrangement produces a position effect phenotype causing various abnormalities of the eye (HINTON and ATWOOD 1941; HINTON 1950). Preliminary investigations suggested that there was a correlation between the requirement and the position effect (HINTON and ELLIS 1950). Subsequent investigations indicated that if a correlation exists, it is not a direct one (HINTON, ELLIS, and NOYES 1951). It was also reported that the requirement usually, but not always, behaved as a dominant effect. This unorthodox behavior was investigated further (HINTON and ROBERTS 1952) and confirmed.

TECHNIQUE

The media used in the various experiments reported here were prepared as described previously (HINTON, NOYES, and ELLIS 1951) except that the basal medium (table 1) contained no nucleic acid or nucleic acid derivatives. In addition, some experiments were performed using a basal medium prepared by the Difco Laboratories and patterned after medium no. 4 as reported by HINTON, NOYES, and ELLIS (1951). Adenine (as ribose adenylic acid or ribonucleic acid) was added to these when necessary for control series.

Since the second chromosome inversion carried by the requiring strain is lethal when homozygous, it is balanced using the "Curly" inversion (*In(2L)Cy*, *In(2R)Cy*). Due to the lethals involved, 50% of the eggs fail to develop into active larvae. In order to eliminate the necessity for inoculating twice the number of eggs needed to

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TABLE 1
Basal medium

	Mg/Ml		Micrograms/Ml
L-arginine	0.559	biotin	0.02
L-cysteine	0.480	B ₁₂	0.028
L-glutamic acid	4.418	Ca-pantothenate	6.0
glycine	1.745	choline chloride	75.0
L-histidine	0.484	pteroyl glutamic acid	36.0
L-isoleucine	1.260	pyridoxine	3.0
L-leucine	2.345	riboflavin	2.4
L-lysine	1.337	thiamine	1.5
DL-methionine	0.339	niacinamide	10.0
L-phenylalanine	1.008		<i>Mg/Ml</i>
DL-threonine	0.756	MnSO ₄ ·4H ₂ O	0.0129
L-tryptophane	1.745	MgSO ₄ ·7H ₂ O	0.246
DL-valine	1.355	FeSO ₄	0.0129
sucrose	7.5	KH ₂ PO ₄	0.606
cholesterol	0.4	K ₂ HPO ₄	0.606
agar	20.0	NaCl	0.0129
		CaCl ₂	0.0129

produce a given number of larvae, the following procedure was used: adult flies were taken from standard *Drosophila* cultures and placed in empty half-pint milk bottles which were inverted over flat dishes containing cornmeal, molasses, agar, and growing yeast. The adult females were allowed 12 to 18 hours to deposit eggs on this mixture. At the end of the egg-laying period the eggs were picked or brushed from the surface of the collecting dishes and placed in small baskets made of fine copper mesh. The baskets were then transferred through a series of washes with Ringer's solution and finally 70% alcohol where they remained for 45 minutes. This treatment has proven adequate to free the eggs from all bacteria and spores and yet not injure the eggs. Using sterile technique, each basket was placed into a small sterile petri dish containing a layer of agar gel. Each basket contained approximately 50 eggs. The eggs were allowed 24 hours to hatch. After the young larvae emerged from the eggs they would usually crawl through the basket onto the surface of the sterile agar. It was a simple matter to remove the larvae, on the end of a wire loop, and transfer them from the petri dish to previously autoclaved test tubes of synthetic medium. Standard bacteriological techniques were used throughout. About 20 larvae were transferred to each test tube.

The results were tabulated in terms of the percent of larvae inoculated that pupated, the average time in days required to reach the pupal stage, and the percent of the pupae which eclosed. The average time to pupation represents the most sensitive criterion for measuring the effect of the medium on growth.

CONTROL DATA

The wild-type strain (Oregon-R), used throughout, does not require adenine for complete growth and development, but growth proceeds at a slower rate when it is omitted from the medium. This is shown by the data in table 2. In the case of Ore-

TABLE 2
Typical examples of growth of the controls

Strain	Medium	Adenine in medium	Original no. larvae	Percent larvae to pupate	Av. time to pupation in days	Percent pupae to become adults
Ore' R	lab	yes	108	97.2	12.5	97.2
		no	96	84.4	17.9	96.3
Ore' R	Difco	yes	57	100.0	11.8	56.1
		no	232	85.3	16.9	(2)
<i>In(2LR)40d/Cy</i>	lab	yes	40	80.0	13.7	93.7
		no	280	9.7* av.	21.5-26.0	(22)
		yes†	146	94.5	14.3	95.6
		no†	123	6.5	21.9	(7)
<i>In(2LR)40d/Cy</i>	Difco	yes	32	62.5	12.4	100.0
		no	170	0.0	—	0.0

* Experiment divided into 7 parts in order to see variability: 0.0, 4.4, 2.2, 14.6, 2.1, 18.7, 25.6.

† A stock made from adults that appeared on a no-nucleic acid medium.

gon-R without adenine, pupation is delayed to around the 17th day, whereas in the *In(2LR)40d/Cy* strain usually no pupae are formed, but this is variable (table 2). When an occasional larva lives long enough to pupate, pupation does not occur until after the 20th day (table 2).

The medium manufactured by Difco allows no pupation in the *In(2LR)40d/Cy* strain unless adenine is added to it (table 2). Therefore most of the experiments were run using Difco medium. If doubt existed as to the validity of earlier experiments they were repeated on Difco medium.

A stock of *In(2LR)40d/Cy* was obtained from the exceptional flies that had developed on medium devoid of adenine. When growth of the new stock was tested, with and without adenine, there was no increase in the percentage pupation (table 2), indicating that genetic modifiers or reverse mutation are not responsible for the occasional survivor.

It should be noted that Ore'-R has a lowered adult hatch when raised on the Difco medium supplemented with adenine. Practically no adults emerge when adenine is omitted. This is despite the fact that the percent pupation is as high as when the medium is made in our laboratory, and adults form within the pupal case. The point is pertinent to some data brought forth by the experiments to be reported here.

RESULTS AND ANALYSIS

The first crosses were made in order to observe the segregation of the requirement with other marked chromosomes so that it might be located as to linkage group. Thus *In(2LR)40d/Cy* (the strain having the requirement) was crossed to a wild-type strain, Oregon-R. Eggs were collected and larvae inoculated onto media part of which contained adenine and part of which did not. Two types of flies would be

TABLE 3

*Offspring from the cross: In(2LR)40d/Cy × Ore'-R**

Adenine	Original no. larvae	Percent pupation	Av. time to pupation in days	Percent pupae becoming adults	No. adults classified	
					Inv.	Cy
Yes	438	94.8	12.0	93.0	—	—
No	363	81.5	17.5	82.8	116	116

* The combined results of 3 experiments.

expected to develop—half should have the rough eye characteristic of the *In(2LR)40d*; half, the curly wing characteristic of *Cy*. However, if the requirement is located on the *In(2LR)40d* chromosome, without adenine only Curly flies should develop; or only straight winged flies if the requirement is on the *Cy* chromosome. It might be expected that the requirement would be found on one of the second chromosomes since they are the only chromosomes characteristic of the strain, the other chromosomes having been replaced several times in outcrossing.

The results of the cross to wild-type were unexpected. Both straight wings and curly wings appeared among the offspring and with an equal frequency, even when raised without nucleic acid. The results from three separate experiments are presented in table 3. There was no significant difference when the female parent was from the Oregon strain or from the *In(2LR)40d* strain.

Not only did both types survive without adenine, but the average time to pupation was the same as it was for the non-requiring wild-type (see table 2). It can be concluded from this that neither of the second chromosomes from the requiring strain expresses the requirement when heterozygous with wild-type second chromosomes.

To see if the requirement would return when the original second chromosomes were brought back together, the offspring from the preceding cross were mated together (*Cy* of one sex × *In(2LR)40d* of the opposite sex). Since both types of flies were heterozygous for wild type chromosomes, four types of second chromosome combinations would be expected in the offspring: wild/wild, wild/*Cy*, wild/*In(2LR)40d*, *In(2LR)40d/Cy*. One of these represents the original combination of second chromosomes characteristic of the strain with the adenine requirement. The results of four such crosses were inconclusive, since the figures were small and some of the other classes were as small as the class under inspection.

In light of these inconclusive data it was deemed best to try a different approach. Therefore, a series of stocks was synthesized representing various combinations of second and third chromosomes from the Oregon wild and the strain with the requirement, and in various cytoplasms.

The stocks synthesized are listed below. The following abbreviations and symbols were employed to refer to the chromosomes: *Inv*—the second chromosome carrying the Inversion (*2LR*)40d; *Cy*—the 2nd chromosome with the Curly inversions; *Ore'*—the wild-type 2nd chromosome from the Oregon-R stock; *I*—the unmarked 3rd chromosome from the stock of *In(2LR)40d/Cy*; *O*—3rd chromosome from the Oregon-R stock; *Bl*—unmarked 3rd chromosome from a stock of Bristle Lobe/*Cy*.

Stocks synthesized

Stock code no.	Chromosomes		Cytoplasm from	Notation
	2nd	3rd		
R ₁	<i>In/Cy</i>	<i>I/I</i>	<i>In(2LR)40d/Cy</i>	Combination recovered following an outcross to <i>Cy/Pm; D/Sb</i> .
D ₄	<i>In/Cy</i>	<i>I/I</i>	<i>Cy/Pm; D/Sb</i>	
R ₄	<i>In/Cy</i>	<i>I/I</i>	Oregon-R	
R ₂	<i>In/Cy</i>	<i>O/O</i>	Oregon-R	
D ₂	<i>In/Cy</i>	<i>Bl/Bl</i>	<i>Cy/Pm; D/Sb</i>	
R ₃	<i>Ore'/Ore</i>	<i>I/I</i>	<i>In(2LR)40d/Cy</i>	

The stocks were synthesized using the technique of crossing flies bearing desired chromosomes to a stock containing 2nd and 3rd chromosomes which were marked by dominant traits and crossover suppressors. By proper crosses, the desired combinations of chromosomes were brought together and the dominant markers eliminated. The dominant marker stock used was *Cy, sp²/Pm, dp b, DcxF, ru h ca/In(3R)Mo, Sb*.

The newly synthesized stocks were next raised on the synthetic medium in order to learn which combinations of the chromosomes and cytoplasm manifested the adenine requirement. The results are summarized in table 4.

Taking only the percent pupation as the criterion for the presence or absence of the adenine requirement, it can be seen that the 2nd chromosome and not the 3rd is necessary for the requirement to be expressed. In the case of R₂ and D₂, where the 3rd chromosomes of the requiring strain have been replaced by 3rds from the Oregon and *Bl L* stocks respectively, the percent pupation remains close to zero. In fact, the second chromosomes are the only genetical factor shared in common by R₂, D₂, and the original requiring strain. Conversely, when the 3rd chromosome and cyto-

TABLE 4
The adenine requirement of stocks R1-4 and D2-3

Stock	Adenine in medium	Original no. larvae	Percent larvae to pupate	Av. time to pupation in days	Percent pupae to become adults
R ₁	yes	63	71.4	15.4	100.0
	no*	299	33.1	23.1	97.0
R ₂	yes	23	73.9	13.5	0.0
	no	89	0.0	—	0.0
R ₃	yes	42	85.7	11.6	97.2
	no	103	59.2	17.6	98.3
R ₄	yes	103	66.0	13.3	75.0
	no	221	14.0	20.8	38.7
D ₂	yes	84	88.1	13.7	86.5
	no	196	1.5	17.0	(1)
D ₃	yes	74	55.4	14.3	80.5
	no	209	4.3	21.6	(3)

* Medium was such that some larvae with the requirement will pupate in the absence of adenine, but not until after the 20th day, as discussed in the section on TECHNIQUE.

plasm of the original strain are left unaltered, but the 2nd chromosomes are replaced (R_3), the requirement is lost.

R_1 , D_3 , and R_4 are alike in respect to the second and third chromosomes, but have different cytoplasms. There is some pupation in each case regardless of the medium used. Although this may not be significant it is possible that during the outcrossing, from which R_4 and D_3 were derived, genetic modifiers were acquired. On the other hand, R_2 and D_2 had a similar origin, yet they do not pupate on the Difco medium. The one thing that R_4 and D_3 have in common which differs from that which R_2 and D_2 have in common are the third chromosomes. R_4 and D_3 both have third chromosomes from the original *In(2LR)40d/Cy* stock while R_2 and D_2 both lack such third chromosomes. This suggests that the third chromosome in the original requiring strain carries modifiers of the requirement which can operate when in certain cytoplasm or when the flies are grown on certain media.

Thus it seems fairly certain that the basis for the adenine requirement is a condition of a second chromosome in the stock *In(2LR)40d/Cy*. The third chromosomes of this stock may possibly carry factors capable of modifying the requirement under certain conditions.

Finally, it should be noted (table 4) that in the case of stock R_2 , no adults appeared either in the presence or absence of adenine. In part this is reminiscent of the behavior of Oregon-R on the Difco medium (table 2). There, no adults appeared when adenine was omitted from the medium even though they did appear when it was added. The only thing unique about R_2 , in comparison with Oregon-R, is that the second chromosomes have been replaced by seconds from the *In(2LR)40d/Cy* stock. This suggests that the second chromosome of Oregon-R is able to perform some vital function, when adenine is in the diet, that neither the third of Oregon nor the seconds of *In(2LR)40d/Cy* can perform. Thus in the combination of seconds from *In(2LR)40d/Cy* and thirds from Oregon, this system concerned with adult eclosion fails to function regardless of the adenine.

Before it was realized that the *In(2LR)40d/Cy* stock required adenine, HINTON (1949) studied the eye phenotype characteristic of the *In(2LR)40d* chromosome. The variable degree to which the eye is affected was measured by distribution curves. It was found that various factors could shift the malformation in one direction or the other. When *In(2LR)40d/Cy* was crossed to Oregon-R and the offspring heterozygous for the inversion and Ore'-R were studied, it was found that the distribution curve had shifted radically toward the extreme effect. This was not true in the case of some of the other crosses made, the curve remaining stationary. The latter was particularly true in the case of a cross to a stock *Bl L²/Cy* (Bristle, Lobe) when the *In(2LR)40d/Cy* offspring were compared with the *In(2LR)40d/Cy* parental stock. Further work was presented to show that the presence or absence of the *Cy* chromosome was not responsible for the change in degree of effect and it was concluded that some third chromosome factor was acting to modify the phenotype.

Since the Oregon-R stock modified both the effect upon the eye and the adenine requirement, and if these two are related, it should follow that the *Bl L²/Cy* stock should not modify the adenine requirement, since it did not modify the eye effect. Therefore a study was made of the adenine requirement of the offspring of a cross of

TABLE 5

Offspring from four crosses of Bl L²/Cy × In(2LR)40d/Cy raised without adenine

	Original no. larvae	Number of pupae by 21st day	Number of adults
1	70	3	1
2	23	1	0
3	390	0	0
4	177	3	2

Bl L²/Cy × In(2LR)40d/Cy. Three types of offspring would be expected: *Bl L²/In(2LR)40d*, *Bl L²/Cy*, *In(2LR)40d/Cy*. However, practically no offspring of any type survived without adenine (table 5), although all three types were obtained in large number in the control with adenine.

The results indicate that not only does the requirement remain after this outcross (as opposed to the results when *Ore'-R* was used in the outcross) but that the *Bl L²/Cy* stock also requires adenine.

In order to obtain further evidence in this connection, *In(2LR)40d/Cy* was crossed to *Ore'-R* and the noncurly offspring (*In(2LR)40d/Ore'*) were selected and mated to *Bl L²/Cy*. Four types of offspring would be expected: *In(2LR)40d/Bl L²*, *In(2LR)40d/Cy*, *Bl L²/Ore'*, *Cy/Ore'*. Without adenine in the medium the only type that survived was the *Cy/Ore'* which is the only type lacking both the *In(2LR)40d* chromosome and the *Bl L²* (17 obtained from 227 larvae). This confirms the results of the previous crosses and further shows that the requirement on the *Bl L²* chromosome is dominant to Oregon.

Examining all of the foregoing evidence, the suggested conclusion is that the basis of the adenine requirement in the *In(2LR)40d/Cy* strain is genetically characteristic of the *In(2LR)40d* second chromosome, is dominant to the Curly second chromosome, but is recessive to the Oregon second chromosome. If this is true, then the question arises as to whether the genetic basis of the requirement is a mutant gene, a position effect of the chromosomal inversion which the strain contains, or some other factor.

In an attempt to distinguish between these possibilities, a study was made of the adenine requirement of a series of stocks which were described by HINTON (1950) and which represent various cytological alterations of the original *In(2LR)40d*. In all of these stocks one end of the original inversion, or both ends, had been involved in a subsequent inversion, translocation, or reinversion. The earlier analysis of these stocks established that the eye effect was a position effect of the inversion. If the heterochromatin located at either end of the inversion were removed to other positions in the chromosome complement by means of these induced aberrations, the eye effect would usually disappear.

Therefore it would seem logical to expect that, if the adenine requirement and the eye effect were both position effects of the inversion, both would disappear upon alteration of the original inversion in some of the cases. A series of these stocks was raised on media lacking adenine. The results are given in table 6.

From the results in table 6 it appears that regardless of the chromosomal arrangement (and the appearance of the eye) the requirement for adenine persists. However,

TABLE 6

*The adenine requirements of various stocks representing cytological alterations of
In(2LR)40d*

Stock	Adenine in medium	Original no. of larvae	Percent larvae* to pupate
In(2LR)IIA	yes	245	69.8
	no	366	8.2
T(2:3)IIG	yes	12	83.3
	no	16	6.2
In(2LR)IIP	yes	88	63.6
	no	230	0.4
T(2:3)IIAH	yes	13	84.6
	no	13	0.0
In(2LR)IIBI	yes	69	59.4
	no	183	1.1
In(2LR)IICA	yes	50	46.0
	no	159	3.8
T(2:3)IICK	yes	34	88.2
	no	87	25.3
In(2LR)IICQ	yes	36	75.0
	no	89	1.1
In(2LR)IICR	yes	23	73.9
	no	34	5.9
T(2:3)IICS	yes	19	78.9
	no	52	0.0
In(2LR)IICX	yes	46	60.1
	no	94	8.5
IICZ	yes	46	58.6
	no	185	0.0
T(2:3)IIDA	yes	32	75.0
	no	70	5.7
IIDC	yes	40	20.0
	no	92	0.0
In(2LR)IIDD	yes	54	66.6
	no	147	2.1
In(2LR)IIDE	yes	24	70.8
	no	103	7.8
T(2:3)IIDG	yes	36	72.2
	no	97	10.3
T(2:3)IIDJ	yes	78	67.9
	no	174	2.9

* The figures for the percent pupae to become adults are not given since they were consistently close to 100% and therefore are of no significance here.

in some cases, a few individuals reached the adult stage with adenine absent from the medium. These cannot be explained as being due to the type of medium used since the Difco medium, which allowed no growth of *In(2LR)40d/Cy*, was used throughout. It may be noted that the percentage of larvae to pupate is quite variable from stock to stock. Seven of the stocks showed no or practically no pupation while the others ranged from 2.1% up to 25.3%. There appears to be no correlation between the amount of pupation and the appearance of the eye or the type of chromosomal rearrangement.

DISCUSSION

It can be concluded that the genetic control for the adenine requirement resides on the same second chromosome as the inversion (*In(2LR)40d*). However, its exact nature has been more difficult to determine.

The original supposition that the requirement may have resulted as a position effect due to the chromosomal inversion is made untenable by the results obtained from testing the series of reversion stocks. These stocks represent cases of cytological alteration of the original inversion accompanied by the disappearance or partial disappearance of the eye effect. The eye effect may be considered a position effect of the inversion which disappears if the heterochromatin at either end of the inversion is removed. Since in no case, of the 18 different tested, had the requirement disappeared, it would follow that the requirement and the eye effect are not being caused by the same agent.

The very fact that the requirement remains in all of the reversion stocks makes the second supposition unlikely, namely that it is due to a gene mutation. If the requirement were due to a point mutation somewhere along the second chromosome it would have had ample opportunity to become lost from some of the stocks through crossing over, because of the very nature of the chromosomal arrangements and the method used to balance the stocks originally. Especially would this be true in the case of the translocations between the second and third chromosomes since they were maintained for many generations heterozygously with cytologically normal chromosomes marked with a second and a third chromosome recessive mutant (*vg; Hn^{r1}*). This method of maintaining the stocks had to be abandoned because the recessive genes in question crossed over onto the translocated chromosomes with such a high frequency as to render them useless as marker genes.

One of the initial breaks which produced the original inversion was located in a heterochromatic region comprising roughly one-third of the mitotic chromosome (HINTON 1941). This region does not expand in the formation of the salivary gland chromosomes and therefore cannot be seen and studied there. Since all cytological analyses were made using only salivary gland chromosomes, one does not know how much heterochromatin was carried to the left end of the inversion or how much remained at the right end. However, it seems definite that a relatively large section is involved at each end of the inversion since, upon irradiating further, breaks occurred at these two places with a too high frequency (HINTON 1950). It would appear that these breaks may occur anywhere within these large regions of heterochromatin and not necessarily at the exact site of the original break. Thus when a subsequent rearrangement occurs, the heterochromatin may become further subdivided instead of being moved as a block. This would also be the most likely situation in the cases which appear to be cytologically complete reinversions.

In fact, the only thing held in common by all of the stocks which have maintained the requirement is the displacement of heterochromatin from its original position in the wild-type. If this particular block of heterochromatin cannot function properly in adenine synthesis when moved from its normal position, or when subdivided, then it would not be surprising that all stocks in the reversion series still show the requirement for adenine. This is to suggest that some step in the synthesis of adenine is controlled by a region of heterochromatin and that the requirement under study

is due to a failure of that region of heterochromatin to function. This is the same region of heterochromatin which exerted the position effect just discussed.

The failure to obtain adults, in certain cases, indicated the presence of additional systems concerned with nucleic acid synthesis and utilization. These cases have been explained in purely hypothetical terms. The essential feature of the analysis is that the third chromosome of the Oregon-R strain apparently carries a factor which makes it difficult for the strain to "store" enough adenine (or perhaps nucleic acid) to last throughout the non-feeding pupal stage. The amount synthesized depends upon certain dietary conditions. The Oregon-R strain can accumulate enough to complete its development only when it is both capable of synthesizing the compound and obtaining extra amounts from the medium. The inversion strain lacks the third chromosome factor and therefore can complete its development from the adenine in the medium, even though it cannot synthesize any due to the presence of the second chromosome. Thus the combination of this third chromosome factor from Oregon-R with the second chromosome of the inversion strain prevents the completion of development even though adenine is present in the medium, as demonstrated by strain R_2 .

It may well be that this third chromosome factor found in Oregon-R is the factor that enhanced the eye effect reported earlier. The degree of effect upon the eye is significantly more extreme both in the inversion offspring from the cross of $In(2LR)40d/Cy \times Ore'-R$ and in the flies from the strain R_2 which have Oregon third chromosomes.

The relation of the adenine requirement to the position effect phenotype as measured by the disturbance in eye formation can now be considered. Our hypothesis which grows out of the foregoing data and discussion is as follows: The original chromosomal rearrangement removed part of a relatively large section of heterochromatin from its normal position near the kinetochore. In its original position and condition this region of heterochromatin served in the synthesis of adenine. As a result of the original chromosomal rearrangement, part of this section of heterochromatin came to lie adjacent to euchromatic genes in the distal part of the left arm of the chromosome, and reciprocally, euchromatic genes from the distal part of the arm came to lie next to that part of the heterochromatin which remained in its normal position. The altered heterochromatin has a general influence on the adenine metabolism of the cell as well as a localized influence on the functioning of the euchromatic region immediately adjacent to it. This influence on the adjacent euchromatin was observed as a malformed eye. Any further disturbance of nucleic acid synthesis or utilization causes the euchromatin adjacent to the influencing heterochromatin to function still less efficiently. When either the distal section of heterochromatin is moved by subsequent chromosomal rearrangement to a new location or when the euchromatin adjacent to the section of heterochromatin remaining at the kinetochore is moved, the localized influence of the heterochromatin disappears. This suggests that the euchromatic region was originally divided into two parts, either part being capable of functioning as the whole when away from the influence of the heterochromatin. The altered heterochromatin, on the other hand, does not function normally in the synthesis of adenine, regardless of its position on the chromosomes. It is not known whether this heterochromatin, in other positions,

exerts an influence on the adjacent chromatin since tests have only recently been started to detect such a situation.

SUMMARY

A strain which has an inherited requirement for adenine was studied in an attempt to identify the genetic basis responsible.

When the strain with the requirement was crossed to a wild-type lacking any requirement for adenine, none of the offspring showed a requirement. When these offspring were inbred and their progeny studied, the data suggested that the requirement was regained only by those individuals with the original second chromosome pair from the adenineless strain, but the results were not definite.

A series of stocks was synthesized representing various combinations of second and third chromosomes from the wild-type and the strain with the requirement. Only those stocks which carried the second chromosomes of the requiring strain proved to have the requirement.

Since the two second chromosomes of the requiring strain are different both cytologically and genetically, it was necessary to make further crosses in order to identify the requirement with one or the other. The results showed that the requirement followed the chromosome which has an inversion known as *In(2LR)40d*.

The various crosses showed that the requirement behaved in a recessive manner to the wild-type but in a dominant manner to second chromosomes from certain other strains.

Attempts were made to separate the requirement from the chromosomal inversion by studying a series of cytological alterations of the inversion. Eighteen different cases were studied and it was found that in no case was the requirement lost. These 18 strains and *In(2LR)40d*, from which they were derived, have in common only the factor of transposed heterochromatin brought about through the original inversion and further transposed by the subsequent alterations. Therefore, it is suggested that the genetic basis of this nutritional requirement lies in the heterochromatin and is a property of the altered heterochromatin itself.

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