3 S.H.A. Malak, Planta med. *29,* 80 (1976).
4 H. Faulstich, D. Georgopoulos, M. Bloching and Th. Wieland, Z. Naturforsch. *29,* Part C, 86 (1974).
5 R.G. Benedict and L.R. Brady, Lloydia *30,* 372 (1967).
6 H. Faulstich and M. Cochet-Meilhac, FEBS Lett. *64,* 73 (1976).
7 A. Gauhe and Th. Wieland, Justus Liebigs Ann. Chem. 859 (1977).
8 W. Burgermeister, Th. Wieland and R. Winkler, Eur. J. Biochem. *44,* 305 (1974).
9 Th. Wieland, H. Faulstich, W. John, M.V. Govindan, H. Puchinger, Z. Kopitar, H. Schmaus and A. Schmitz, Hoppe Seylers Z. Physiol. Chem. *353,* 1337 (1972).
10 H. Faulstich, Th. Wieland, A. Walli and K. Birkman, Hoppe-Seylers Z. Physiol. Chem. *355,* 1162 (1974).
11 A.M. Lengsfeld, I. Low, Th. Wieland, P. Dancker and W. Hasselback, Proc. nat. Acad. Sci. USA *71,* 2803 (1974).
12 Th. Wieland, Naturwissenschaften *64,* 303 (1977).
13 J. Wehland, M. Osborn and K. Weber, Proc. nat. Acad. Sci. USA *74,* 5613 (1977).
14 P. Chambon, A. Rev. Biochem. *44,* 613 (1975).
15 R.G. Roeder, in: RNA Polymerase, p.285 R. Ed. R. Losick and M. Chamberlin. Cold Spring Harbor Laboratory, 1976.
16 L.G. Hanelin and A.A. Moss, Am. J. Roentg. *125,* 782 (1975).
17 L. Fiume, V. Marinozzi and F. Nardi, Br. J. exp. Path. *50,* 270 (1969).
18 L. Fiume, M. Derenzini, V. Marinozzi, F. Petagzi and A. Testoni, Experientia *29,* 1520 (1973).
19 M. Morrison and G.R. Schonbaum, A. Rev. Biochem. *45,* 861 (1976).
20 P.W. Morris, D.L. Venton and K.M. Kelley, Biochemistry *17,* 690 (1978).
21 H. Musso, in: Oxidative Coupling of Phenols, p.1. Ed. W.I. Taylor and A.R. Battersby. Marcel Dekker, Inc., New York 1967.
22 B.R. Brown, in: Oxidative Coupling of Phenols, p.167. Ed. W.I. Taylor and A.R. Battersby. Marcel Dekker, Inc., New York 1967.
23 U. Fauser and H. Faulstich, Dt. med. Wschr. *98,* 2259 (1973).

Position effect influencing alcohol dehydrogenase activity in *Drosophila melanogaster*[1]

B.N. Hisey, J.N. Thompson, Jr, and R.C. Woodruff

Department of Zoology, University of Oklahoma, Norman (Oklahoma 73019, USA), and Department of Biological Sciences, Bowling Green State University, Bowling Green (Ohio 43403, USA), 31 July 1978

Summary. A small decrease in alcohol dehydrogenase activity was found in translocation stocks of *Drosophila melanogaster* in which a section of the heterochromatic Y-chromosome is inserted proximal to *Adh.* This small position effect is consistent with our growing knowledge of the control and transcription of the *Adh* locus.

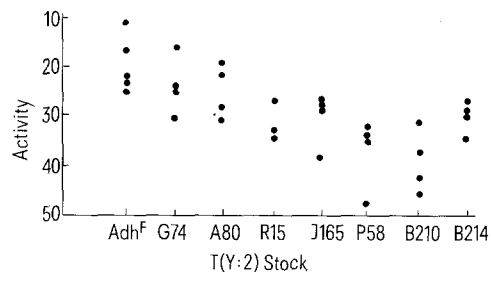
Position effect can be defined as the correlation between a gene's location and its expression, which can be influenced by its proximity to other genes and to heterochromatin. Position effects have been shown for a variety of morphological traits[2] and even for enzyme loci, such as amylase *(Amy)* in *Drosophila melanogaster.* Because of the growing interest in alcohol dehydrogenase as a model genetic system, we were interested in learning whether the activity of the *Adh* locus could be affected by its proximity to heterochromatin.

Alcohol dehydrogenase (ADH) is a small homodimer coded for by a gene on the left arm of the second chromosome (2-50.1) of *D. melanogaster.* It has been localized cytologically by Woodruff and Ashburner[4] to polytene chromosome bands 35B2-3. ADH activity can be measured spectrophotometrically by the rate at which the supernatant from homogenized whole flies breaks down an alcohol substrate. We prepared the homogenates from 1-3-day-old adults by weighing 40 mg of flies or by counting out 40 flies of each sex. Both types of homogenates gave the same results. Activity assays were run by placing 0.1 ml of the homogenized fly supernatant in 2.4 ml of a buffered solution containing 0.072 M tetrasodium pyrophosphate, 0.072 M semicarbazide HCl, 0.021 M glycine and 0.065 M sodium hydroxide in distilled water, with 4 mg β-NAD and 0.03 ml of isopropanol as the substrate. Activity was determined from a plot of the change in absorbance at 340 nm versus time.

In order to monitor *Adh* position effects, we assayed the relative enzyme activities in a number of T(Y : 2) translocation stocks. In these the heterochromatic Y-chromosome was attached to chromosome 2 at various distances to the left and the right of *Adh.* These stocks were homozygous for the Adh^F (fast electrophoretic mobility) allele.

ADH activity assays of 40 mg of flies from 7 different T(Y : 2) stocks[5] are shown in the figure, with translocation breakpoints given by Lindsley et al.[5]. It is clear from this figure that there are repeatable differences in activity from stock to stock. Thus, since the stocks are believed to be genetically identical, except for the Y and chromosome 2 breakpoints, it appears that ADH activity can be influenced by either the amount or proximity of heterochromatin.

The results of detailed assays of four translocation stocks are shown in the table. In these assays, we have corrected for fly size, since ADH activity is apparently not directly correlated with adult b.w[6]. We have also eliminated the possibility that additional Y-chromosomes are present. As in the figure, it can be seen that the activity of stock P58, in which the Y-heterochromatin is inserted proximal to *Adh,* is consistently lower than the activity of strains with heterochromatin inserted distal to *Adh.* Although the amount of Y-heterochromatin differs among stocks G74, A80 and P58, the makeup of stocks P58 and B210 appears to be about the same. Yet the ADH activity is higher in B210 than in P58, in which the breakpoint and proximity of



ADH activities in 7 T(Y:2) translocation stocks of *Drosophila melanogaster.* The activity scale is a temporal one, based upon the time (in sec) required to change the OD a standard amount in a reaction catalyzed by ADH. Thus, lowest activities require the longest time (50) and highest activities the shortest time (less than 10 sec).

Mean and SD for relative ADH activities of 4 T(Y:2) translocation stocks

	G 74	A 80	P 58	B 210
Females	0.256±0.068	0.238±0.047	0.209±0.045	0.227±0.047
Males	0.204±0.052	0.219±0.063	0.168±0.059	0.217±0.068
Total	0.230±0.063	0.228±0.053	0.188±0.054	0.222±0.055

N = 10. Pooling male and female data, $p \leq 0.05$ for all comparisons of stock P58 versus the other stocks.

heterochromatin is nearer to *Adh*. Fly sizes were similar in each strain, though some part of the difference in relative activities could be due to minor position effects or genetic differences affecting fly size. Whether due to amount or to the relative position of heterochromatin, however, the apparent position effect upon *Adh* is small. Thus, these experiments should be repeated with the more effective centromeric heterochromatin when appropriate stocks become available.

Finally, it is interesting to note that these observations are consistent with our growing knowledge of the control and transcription of the *Adh* locus. A presumptive control mutation affecting ADH activity has been isolated by Thompson, Ashburner and Woodruff[7] and mapped proximal to *Adh*. This is consistent with the position effect data showing decreased activity, perhaps through inactivation of a control locus, when heterochromatin is inserted proximal to *Adh*.

1 We are grateful to Dr D.L. Lindsley who provided the T(Y:2) stocks to R.C.W. and to Dr J.F. Halsey for his advice and hospitality in his laboratory. This research was supportee, in part, by a Faculty Research Council grant from the University of Oklahoma to J.N.T.

2 J.B. Spofford, in: The Genetics and Biology of Drosophila, vol. 1c, p. 955. Ed. M. Ashburner and E. Novitski. Academic Press, London 1976.
3 E. Bahn, Hereditas *67*, 79 (1971).
4 R.C. Woodruff and M. Ashburner, unpublished manuscript.
5 D.L. Lindsley, L. Sandler, B.S. Baker, A.T.C. Carpenter, R.E. Denell, J.C. Hall, P.A. Jacobs, G.A.G. Miklos, B.K. Davis, R.C. Gethmann, R.W. Hardy, A. Hessler, S.M. Miller, H. Nozawa, D.M. Parry and M. Gould-Somero, Genetics *71*, 157 (1972).
6 B. Clarke, personal communication.
7 J.N. Thompson, Jr, M. Ashburner and R.C. Woodruff, Nature *270*, 363 (1977).

Isostaining and iso-nonstaining in 5-bromodeoxyuridine-substituted *Vicia faba* chromosomes

I. Schubert, P. Döbel and S. Sturelid

Zentralinstitut für Genetik und Kulturpflanzenforschung der AdW der DDR, DDR-4325 Gatersleben (German Democratic Republic), and Department of General Genetics, University Uppsala, S-75007 Uppsala (Sweden), 24 July 1978

Summary. Isostaining and iso-nonstaining was studied by the fluorescent plus Giemsa (FPG) technique after immersion of *Vicia faba* roots in 5-bromodeoxyuridine (BrdUrd) for about 1 cell cycle (17 h) and in thymidine (19 h) for another one. Both phenomena were seldom observed, never occurred together in the same cell, and are interpreted as being due to deviations of the cell cycle duration.

Isolabelling (silver grains observed in both chromatids in certain positions of a chromosome) or iso-nonlabelling (absence of silver grains at a certain position of both chromatids of 1 chromosome) were detected by autoradiography in the course of the 2nd and 3rd mitosis after incorporation of ^{3}H-thymidine into chromosomal DNA by a number of authors[3,10]. Different interpretations of these phenomena have been given:

1. Sister chromatid exchanges (SCEs) on the basis of single strand DNA exchange could be responsible for these phenomena. For kinetic reasons, this seems to be an improbable explanation[3].

2. Somatic crossing-over between homologous chromosomes could also cause the phenomena. The absence of simultaneous occurrence of isolabelling and iso-nonlabelling in homologous chromosomes of the same cell[3], as well as the rather weak correlation between sites of chromatid translocations and SCEs[8], are not in accord with this hypothesis.

3. The presence of true subchromatids and the lack of semiconservative segregation of DNA could be another reason for isolabelling, or 4., as an alternative explanation to 3., isolabelling could be due to autoradiographic image spread at the sites of exchanges of very small parts of chromatin. This was indeed found by Wolff and Perry[9]. As far as we know, Luchnik and Porjadkova[5] were the first to report isostaining, which corresponds to isolabelling, after application of the FPG-technique on γ-irradiated human lymphocytes; they interpreted it as evidence for a polynemic chromosome structure, i.e., for the existence of true subchromatids in chromosomes.

During our studies of the intrachromosomal distribution pattern of SCEs[8] in *Vicia faba*, we occasionally found in the slides from some roots chromosomes with isostaining or iso-nonstaining. Using a slightly modified version of the FPG technique according to Kihlman and Kronborg[4] (for methods used, see Schubert et al.[8]), we normally did not come across these phenomena. Among 159 differentially stained metaphases from 5 different slides in which isostaining and iso-nonstaining occurred, respectively, 14 metaphases (8.8%) showed isostaining and 4 metaphases (2.5%) showed iso-nonstaining. In none of these cases, however, did the 2 complementary situations occur together in the same cell. For this reason, the first 2 interpretations mentioned above may be excluded.

Instead of assuming the existence of true subchromatids, a simple explanation for the exceptional occurrence of iso- or iso-nonstaining, at least in *Vicia faba*, would be a deviation in cell cycle duration (cell cycle data of the karyotype used[6,7]). If BrdUrd treatment for 17 h corresponds to the duration of approximately one cell cycle, and if some cells, which at the beginning of BrdUrd application already have started or just finished DNA replication, need more time (about 21–22 h) for passing a complete cell cycle, then the