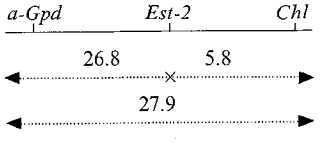
because all Chl^{SS} must be killed by a 24-h exposition to 0.0015 ppm chlorpyrifos.

The results given in table 2 show that *a-Gpd*, *Est-2* and *Chl* are localized on the same chromosome. *a-Gpd* and *Chl* are separated by 27.9 units of crossing-over, *Est-2* and *Chl* by 5.8 units, and *a-Gpd* and *Est-2* by 26.8 units. (*a-Gpd* and *Est-2* were found to be distant of 37.1 recombination units in a previous experiment[5] using SG and S7 strains only. This value is not significantly different from ours, $\chi^2 = 3.69$ for 1 df, $p > 0.05$.) The disposition of the 3 genes is therefore:



Discussion. In *Culex pipiens pipiens* from Southern France, chlorpyrifos resistance is determined by a single gene named *Chl* with 2 alleles, Chl^S which is recessive, and Chl^R dominant. The *Chl* locus belongs to the same linkage group as do the *a-Gpd* and *Est-2* loci and is at some 5 units of crossing-over from the later. *a-Gpd* and *Est-2* loci are known to be on an autosome[4,5]. De Stordeur[4] believes that *Est-2* does not belong to linkage group II because he observed an independant assortment between this locus and the *fc* (yellow larvae) locus generally attributed to the 2nd chromosome[7]. If this information is confirmed, it would mean that the locus inducing chlorpyrifos resistance is on a different chromosome than that bearing the *ma* (malathion resistance) locus in Japanese *Culex pipiens pallens*[8] and the *fe* (fenthion resistance) locus in Burman *Culex pipiens fatigans*[9].

It can be concluded that the *Est-2* locus, when it codes the $Est\text{-}2^{0.64}$ allele is not responsible for chlorpyrifos resistance, and therefore, the highly significant correlation observed between $Est\text{-}2^{0.64}$ frequency and chlorpyrifos resistance in natural populations of Southern France is most probably the result of a tight linkage between 2 independant loci.

The analysis of organophosphate resistant *Culex* of different geographic origins[10] has pointed out that all resistant strains demonstrate an extremely high level of esterase activity. This is not the case of the $Est\text{-}2^{0.64}$. In France, the allozyme demonstrating the highest activity is coded by the *Est-3* locus which is localized at some 3 units of crossing-over from *Est-2*. Experiments are actually underway to localize this last locus with respect to *Chl*.

1 N. Pasteur and G. Sinègre, Biochem. Genet. *13*, 789 (1975).
2 G. Sinègre, J.L. Jullien and O. Crespo, Cah. ORSTOM *15*, 49 (1976).
3 G. Sinègre, J.L. Jullien and B. Gaven, Cah. ORSTOM, in press (1978).
4 E. de Stordeur, Biochem. Genet. *14*, 481 (1976).
5 N. Pasteur and E. de Stordeur, Genetica *46*, 319 (1976).
6 The SG strain is homozygous for $Est\text{-}2^{1.00}$ and segregates for $a\text{-}Gpd^{1.00}$ and $a\text{-}Gpd^{1.40}$; the S5 strain is homozygous for $a\text{-}Gpd^{1.00}$ and $Est\text{-}2^{0.64}$; and the S7 strain is homozygous for $a\text{-}Gpd^{1.00}$ and $Est\text{-}2^{Nul}$.
7 H. Laven, in: Genetics of Insect vectores. Ed. J.W. Wright and R. Pal. Elsevier Publ. Co., Amsterdam 1967.
8 T. Tadano, Jap. J. sanit. Zool. *20*, 158 (1969).
9 C. Dorval and A.W.A. Brown, Bull. W.H.O. *43*, 727 (1970).
10 G. Georghiou and N. Pasteur, J. Econ. Ent., Section B, in press (1978).

Lysine estimation with the modified Udy-dye binding method in hexaploid wheat

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Summary. Lysine content in bread wheat (*Triticum aestivum* L.m. Thell) was determined by a modified Udy-dye binding method and was compared with that obtained from the amino acid analyzer. The values obtained from the 2 methods were correlated and the co-efficient was found to be +0.91 at 0.450 mg/ml dye concentration. The modified method is quick, less expensive and quite helpful for screening lysine at earlier generation in wheat breeding for improved grain quality content.

It has been reported[2-4] that lysine is the most limiting essential amino acid and is present in wheat much below the required amount needed for nutritional balance. About 80% of people in Pakistan and nearly 1 billion people in the world depend on wheat as their main staple food. The green revolution has been effective to a certain extent in meeting the caloric requirements of the underdeveloped countries, but so far no significant achievement have been made in improving the nutritive value of the bread wheat (*Triticum aestivum* L.m. Thell). This is probably due to the fact that the plant breeders in general are unable to screen larger plant population for basic amino acids. Although various methods for screening lysine have been reported[4-8], these methods are slow and time-consuming. Screening lysine with amino acid analyzer is not only slow but also unsuitable for mass screening. Laboratories with meagre financial support must devise less expensive and quick screening methods for lysine determination. A modified Udy-dye binding method for screening barley genotypes was tried with changed dye concentrations for estimating lysine content in hexaploid wheat. The present work deals with lysine screening in bread wheat and the lysine values thus obtained were compared with those determined from the amino acid analyzer.

Material and method. Protein estimation with Udy-method[10,11] is essentially based on the principle that the protein binds quantitatively with certain dyes and each protein has precise dye-binding capacity (DBC). Acid orange-12 (AO-12) when reacting with proteins, binds strongly with basic groups of amino acid, viz lysine, arginine, histidine, and forms insoluble protein complexes. The filtration of these complexes and calibration of unbound equilibrium dye concentration (EDC) through Udy-color analyzer enables us to estimate the protein content.

Slight modification[9] in the Udy-method[11] was made and low dye concentrations of reagent dye, as suggested[7], were used. Accordingly Udy-color analyzer was set at 20% transmission (T) with reference dye instead at 42% (T) to provide a wider range for calibration. At this setting 30% T was observed with 0.450 mg/ml or diluted dye concentration. Wheat flour of 9 wheat cultivars, with known analyzer lysine values, were reacted with the following reagent dye concentrations: 0.325, 0.350, 0.375, 0.400, 0.425, 0.450, 0.475, 0.500, 0.525 and 0.575 mg/ml. 100 mg of wheat flour

(meal) of each cultivar reacted with 15 ml of the above dye concentrations. The material was shaken (4 min) and filtered (Udy-milling, shaking and filtering equipment). The percentage T (% T) in Udy-color analyzer was recorded and quantity of dye not bound by the sample or meal, i.e. equilibrium dye concentrations (EDC), was calculated as follows:

$$EDC = \frac{\text{diluted dye concentration} \times \%\ T\ (\text{without meal})}{\%\ T\ (\text{with meal})}$$

By substituting values % T (with meal), a standard table was prepared to read EDC values. The average EDC values of individual wheat sample calculated from each of diluted dye concentration were taken as X and analyzer lysine value as Y and regression of X on Y was determined with the help of the regression equation i.e. $Y = A + BX$. The correlation coefficient of EDC with analyzer lysine values were observed +0.96. By substituting values of X, a standard table was prepared to read lysine values.

Results and discussions. It was suggested[7] that the dye concentration of reference or reagent dye (1.3 mg/ml) used in the Udy procedure for protein estimation is too high for lysine screening. Dye concentration lower than 0.50 mg/ml is advisable for lysine screening. In the present work, it was observed that 0.450 mg/ml dye concentrations were most suitable for lysine screening. Therefore the 2 dye concentrations, viz 0.450 and 0.425, selected from the preliminary experiment, were used for lysine estimation. 100 mg of wheat flour, from 12 wheat cultivars, reacted with 15 ml of the 2 diluted dye concentrations. The lysine values calculated with the help of the modified method, described in material and method portion, were compared with the analyzer lysine and their coefficient correlation were observed +0.91 and +0.80 respectively. Since the dye meal ratio used was the same as described[9], the lysine values obtained from the modified method may be compared with analyzer lysine. Later on, 21 wheat cultivars were evaluated (table) for lysine values, using 0.450 dye concentration. The lysine values obtained from the modified method were compared with analyzer lysine and +0.75 correlation was observed. The mean value of the lysine from the 2 methods is more or less identical. However, the slightly lower correlation observed in the data reported may be due to various factors such as room temperature, environmental condition and variation within the sample. Such variations in lysine values have also been reported[7,9]. The results further confirm the validity of DBC method[7] for screening lysine content in cereals, and dye concentration used[9] for estimating lysine values in barley is more or less the same for lysine determination of hexaploid wheat. It is therefore concluded that the modified Udy method is very useful for preliminary lysine screening. It is also quick for mass screening at an earlier generation in wheat breeding programme. In 1 h, about 20-25 samples can be analyzed using extra shaker tray instead of single react-R-tube. After initial scrutiny selected samples may be analyzed with amino acid analyzer.

Lysine values observed with the analyzer and the modified Udy dye binding method

Wheat cultivars 1975 Yuma No.	Protein (%)	Lysine percent of protein	
		Analyzer	Udy-modified method
10,100	16.0	3.3	3.3
11,522	12.1	3.3	3.5
11,574	13.0	3.3	3.5
11,847	13.1	3.4	3.4
11,524	12.9	3.4	3.5
11,511	12.2	3.3	3.5
11,513	13.1	3.3	3.5
11,564	12.9	3.4	3.4
11,074	12.6	3.3	3.4
11,507	13.6	3.4	3.5
10,967	13.4	3.3	3.4
10,491	12.4	3.6	3.6
11,593	14.1	3.4	3.4
10,461	12.5	3.6	3.5
10,841	12.7	3.6	3.6
10,699	12.4	3.6	3.6
10,811	11.8	3.7	3.5
10,451	10.9	3.7	3.6
10,642	11.0	3.8	3.6
10,038	16.0	3.2	3.2
10,121	17.0	3.2	3.2
		3.4	3.5

r = +0.75.

1 The author is grateful to Dr V.A. Johnson, University of Nebraska, Lincoln, USA, for supplying wheat cultivars with known protein and lysine values.
2 M.J. Lewrence, J.M. Day, M. Katherine, H. Edith and Barbara Lee, Cereal Chem. *35*, 169 (1958).
3 E.E. Dermot and J. Pace, J. Sci. Fd Agric. *11*, 109 (1960).
4 E. Villegas, C.E. Mc Donald and K.A. Giles, Am. Ass. Cereal Chem. *47*, 147 (1970).
5 Y. Pomeranz and B.S. Miller, J. off. agric. Chem. *45*, 399 (1963).
6 M.L. Kakade and I.E. Liner, Analyt. Biochem. *27*, 273 (1969).
7 R. Mossberg, in: New approaches to breeding for improved plant protein, p. 151. Int. Atomic Energy Agency, Vienna 1969.
8 F.P. Zscheila, Jr, and B.L. Braman, Analyt. Biochem. *49*, 442 (1972).
9 R.S. Bhatty and K.K. Wu, Can. J. Pl. Sci. *55*, 685 (1975).
10 D.C. Udy, Cereal Chem. *33*, 190 (1956).
11 D.C. Udy, J. Am. Oil Chem. Soc. *48*, 29 (1971).

Down syndrome – transferrin parallels plasma iron changes

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Summary. The transferrin level was studied in patients with simple trisomy 21 and with Robertsonian unbalanced translocations 21/22 and 21/14. In all these groups of patients, known to have significantly lowered plasma iron levels, the transferrin levels were found to be decreased with respect to the control group.

Our previous investigations revealed changes in the plasma protein fractions in patients with Down syndrome manifested by a slight growth of the β_1-globulin fraction[4]. Simultaneously, a distinct decrease in the level of plasma iron was observed in these patients[5]. One can expect that the diminution in the plasma iron may be conditioned chiefly either by a decrease in the transferrin level or by an impaired iron absorption in the digestive tract.