(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.

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Down syndrome – transferrin parallels plasma iron changes

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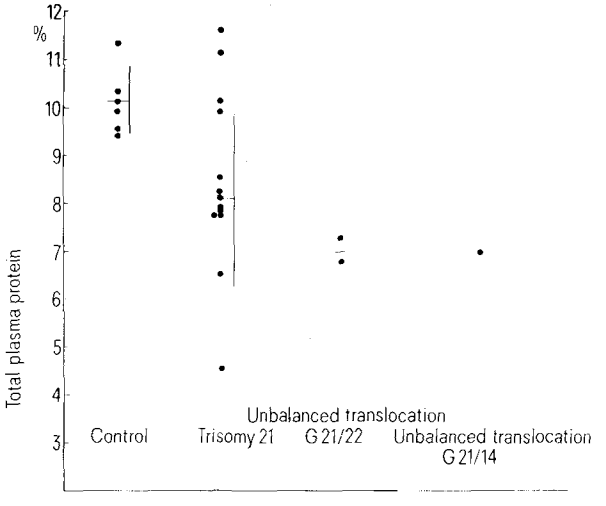
Summary. The transferrin level was studied in patients with simple trisomy 21 and with Robertsonian unbalanced translocations 21/22 and 21/14. In all these groups of patients, known to have significantly lowered plasma iron levels, the transferrin levels were found to be decreased with respect to the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

The aim of this investigation was to analyse the ferritin content with respect to the plasma iron concentration in patients with Down syndrome.

Material and methods. Venous blood was taken from fasting donors into heparinised tubes. The 1st group of donors consisted of 13 patients with trisomy 21 aged from 15 up to 23. Brother and sister aged 26 and 25, respectively, constituted the 2nd group. The 3rd group of donors consisted of 6 healthy individuals of similar age. No infectious disease was found during blood testing in all the donors, and the general health status of the patients with Down syndrome was on the borderline of normal.

Blood plasma proteins were separated in 6% polyacrylamide gel, using a Tris-glycine buffer, pH 8.6[6]. 20 µl of plasma diluted with 10% sucrose (1:10) were applied to the gels. The electrophoresis was run for 45 min, at a current of 4 mA per tube. The protein was stained according to Reisner[7]. All samples were run at least in triplicate. The protein fractions on electropherograms were evaluated in a Vitatron TLD-100 densitometer and the percentage of transferrin was estimated in relation to the total serum protein.



Results. The level of transferrin in patients with trisomy 21 was decreased with respect to the control group (8.3±1.8 vs 10.1±0.7% of the total protein, mean ±SD). This difference was statistically significant ($p < 0.05$). Particularly low transferrin levels were observed in patients with translocations 21/22 (6.7 and 7.2%) and 21/14 (6.9%) (figure).

Discussion. In the cases of Down syndrome due to both trisomy 21 and unbalanced translocations (21/22 and 21/14), lowered levels of relative transferrin levels were found. As no significant changes in the total plasma protein exist in Down syndrome[4], these decreases indicate also corresponding changes in absolute ferritin levels. It is interesting to note that a decrease in the plasma iron in trisomy 21 was established in our previous study[5], and even greater decrease in the cases of translocation 21/22. These findings would suggest a possibility of a causative relationship.

An attempt to interprete the low levels of transferrin in Down syndrome on a genetic background would be difficult. The synthesis of transferrin is regulated by a number of allelic genes. Most people have the Tfc/Tfc genotype[8]. A complete lack of transferrin is a very rare phenomenon, described only in few cases[9]. Lowered transferrin levels are not accompanied by clinical symptoms[10]. The observed decrease in the transferrin level in Down syndrome may involve some dependence on the karyotype anomaly and may be due to highly complex disturbances of metabolic processes of protein turnover in this disease.

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Time-qualified reference intervals – chronodesms[1]

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Summary. Methods are presented for qualifying clinical reference intervals, for individuals as well as peer groups, according to circadian and other rhythms, using chronobiologically-defined single samples or time series.

Circadian and other rhythms are known to characterize many variables of clinical interest[9-11,16,21-23] but are almost completely ignored in compilations of 'normal ranges'[6,17,20]. These rhythms may contribute a large fraction of the variability seen in clinical data. Accordingly, diagnosis could be refined if data were time-coded and interpreted against time-qualified reference intervals, herein called 'chronodesms'[2].

The construction of reference intervals qualified by time along a 24-h scale (i.e. of *circadian* chronodesms) will be described but it should be realized that actual evidence on statistically significant rhythms along several time scales in one and the same variable - such as circulating prolactin - is available and, hence, reference intervals may be qualified in this case on the circannual as well as circadian time-scales.

In the simplest case, assume a set of data consisting of single samples obtained from a reasonably homogeneous group of healthy subjects at approximately the same clock hour. (If clock hour is to be used as the time-marker all subjects must be on similar schedules, at least of wakefulness and sleep.) Because the methods to be used assume a normal (Gaussian) distribution of values, the first step is to test this assumption using the Kolmogorov-Smirnov (K-S) test[4]. Values are first ranked in order of increasing magnitude and each *different* value (y_i) is converted to a normalized deviate, Z_i ($Z_i = (y_i - \bar{y})/s$, where $\bar{y}$ = mean of all values and s = standard deviation). The cumulative *expect-*