Other actinomycins (I, V and C), which differed from AcD in the amino acid arrangement in the cyclic penta-peptide lacton rings but which were thought to inhibit RNA synthesis in a manner similar to AcD, did not affect the EEG even at higher dose (0.5 mg/kg). In mice, AcD of 10 µg and 7-aminoAcD of 10 µg (per mouse) caused neither disappearance of EEG nor spike activity. The effects of AcD and 7-aminoAcD seem species specific.

The findings suggest that the effects of AcD and 7-aminoAcD on the EEG are due to neither nonspecific action involved by injection of the agents nor inhibition of RNA synthesis. Such effects may be related to their direct action on the molecular structure of the neuronal membrane. The agents may also play a role as antagonists to known neurotransmitters. The sites of peptide lacton rings seem to be important for the appearance of the effect on the EEG, although the mechanism of the action is not yet understood.

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Down's syndrome: permeability of the erythrocyte membrane for spin-labeled non-electrolytes[1]

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Summary. A decreased rate of membrane transport of a hydrophobic non-electrolyte TEMPO was found. There were no significant changes in that of a hydrophilic non-electrolyte TEMPOL in erythrocytes of patients with trisomy 21. Changes of the same direction in erythrocyte membrane permeability were found to occur during the intravascular erythrocyte aging.

Considerable evidence indicates that the genetic anomaly of trisomy 21 brings about multiple structural and metabolic disturbances at the cellular level[3,4]. The red blood cell, due to its accessibility and simplicity, has been a subject of many studies in this respect[4,5]. These studies have demonstrated alterations in the ultrastructure and functional properties of the red cell membrane, including changes in the permeability for some amino acids[4] and an abnormal pattern of osmotic fragility[5]. Our recent studies have demonstrated that the ultrastructural investigations on the erythrocyte membrane in Down's syndrome reveal irregularities typical of senescent erythrocytes in normal donors[6]. Spin label studies have pointed to alterations in the structure of membrane proteins[7] similar to those found during cell aging[8]. As the aging of bovine red cells in vivo involves characteristic changes in membrane permeability for spin-labeled non-electrolytes[8], examination of this parameter in erythrocytes from patients with Down's syndrome and comparison with changes observed during intravascular aging of human red blood cells seemed to be of interst.

Material and methods. Erythrocytes of 6 healthy donors were separated according to age using the method of Murphy[9]. Five 'layers' of cells (each making up 20% of the total cell volume) were to be differentiated in the centrifuge tube. The cells of 3 layers were used for this study: the top cells (youngest and lightest), the middle density cells, and the lowest cells (oldest and of greatest density). They were withdrawn and washed 4 times with phosphate-buffered saline. Transport of spin-labeled non-electrolytes 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) across the erythrocyte membrane was studied by the ESR method[10]. Briefly, a small amount of a stock solution of a spin label was added to a red cell suspension of hematocrit 0.50. The initial spin label concentration in the suspension was 100 µM. The sample was mounted in the cavity of an ESR spectrometer and the rate of decay of the ESR signal of a label was monitored at ambient temperature of 21 ± 1 °C. Upon entering the cell interior the labels were reduced to non-paramagnetic derivatives. Studies of kinetics of reduction of the spin labels in question by cell suspensions and hemolysates as well as effects of membrane-modifying agents on this reduction demonstrated that the rate of decay of the ESR signals of the spin labels from erythrocyte

Table 1. Permeation constants of TEMPO and TEMPOL into different age fractions of human erythrocytes (mean ± SD, n = 6); Y, youngest; M, medium; O, oldest cells

Erythrocytes	$k/min^{-1}/\times 10^2$ TEMPO	TEMPOL
Y	2.02 ± 0.25	0.83 ± 0.18
M	1.73 ± 0.15*	0.78 ± 0.12
O	1.49 ± 0.09**	0.70 ± 0.19

Statistical significance of differences with respect to youngest cells: * $p < 0.05$, ** $p < 0.01$ (Student's t-test).

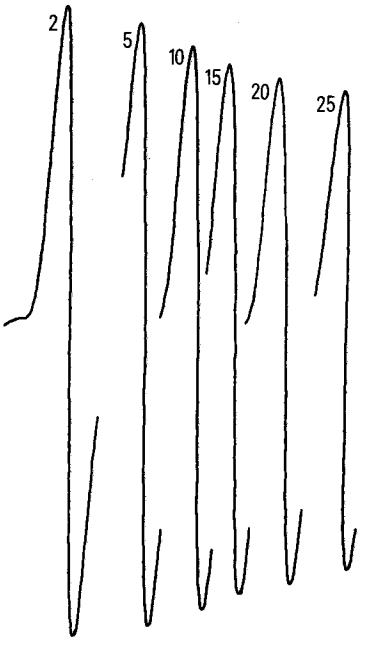
Table 2. Penetration constants of TEMPO and TEMPOL into control and Down's syndrome erythrocytes (mean ± SD, n = 8 and n = 10, respectively)

Erythrocytes	$k/min^{-1}/\times 10^2$ TEMPO	TEMPOL
Control	1.75 ± 0.39	0.75 ± 0.31
Down's syndrome	1.21 ± 0.32	0.71 ± 0.18
Statistical significance of difference	$p < 0.01$	NS

suspension reflects the rate of permeation of the red cell membrane by these compounds[11].

In a parallel experiment, blood was taken from 10 patients with trisomy 21 (karyotpye 47,XX,21+ or 47,XY,21+) and 8 age-matched healthy controls, and transport of both labels into non-fractionated erythrocytes of both groups of donors was compared.

Results and discussion. Reduction of TEMPO by erythrocyte suspension illustrating the principle of the method is shown in the figure. Within the time period employed this reduction obeyed simple exponential kinetics: $c = c(O) \exp(-kt)$ where: c-concentration of a label in the sample (proportional to the height of the ESR signal), t - time, k - penetration constant.



Reduction of TEMPO by human erythrocyte suspension. Intensity of the midfield peak of ESR spectrum as a function of time after introduction of the label (indicated in min).

Comparison of penetration constants of the labels for different age fractions of human red cells (table 1) confirms the results obtained for bovine erythrocytes[8] a decrease in the rate of penetration with increasing cell age. This effect is more pronounced under TEMPO than under TEMPOL.

Comparison of penetration constants for erythrocytes of normal donors and patients with Down's syndrome (table 2) reveals a significant reduction in the penetration rate of TEMPO and an insignificant decrease in the penetration rate of TEMPOL in Down's syndrome. These results are in agreement with the hypothesis[6] proposing that there is accelerated erythrocyte aging in Down's syndrome at the membrane level.

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Multiple overwintering mechanisms in *Chymomyza amoena* larvae (Diptera: Drosophilidae) and laboratory induction of freeze tolerance

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Summary. Chymomyza amoena larvae in apples in summer were found to be either potentially freeze tolerant or to supercool. By late September only potentially freeze tolerant larvae were recovered from apples. Larvae from walnut husks in winter supercooled to avoid freezing. However, freeze tolerance could be induced in laboratory grown larvae by placing them in apples around the seed area and maintaining them at chilling temperatures for several weeks. Overwintering mechanisms employed by *C. amoena* larvae in Michigan appear to depend upon larval feeding site.

Chymomyza amoena in Michigan has been breeding in apples since 1891 in association with primary pests[1,2]. Band and Band[3] found that fly larvae were overwintering in apples and using proteins to achieve cold-hardiness. Hemolymph osmolarity, 665 mOsm, was in agreement[4]. The watery condition of apples collected in March, 1981, after defrosting, suggested that larvae might be freeze tolerant[5]. Freeze tolerant organisms usually have an elevated freezing point, above $-10\,^{\circ}C$, which promotes rapid freezing. Freezing and supercooling points are the same and cold acclimated larvae are able to recover. In summer only potential freeze tolerance is expressed and winter-collected organisms also lose freeze tolerance if kept at room temperature[6-8]. Organisms that rely on supercooling to achieve cold-hardiness remain freeze sensitive though they may produce additional antifreeze agents to lower the freezing point as the season changes from summer to autumn to winter[9]. Unpublished data on larvae grown on chymomyzid medium indicated that *C. amoena* larvae supercooled.

C. amoena larvae feeding in apples in summer and fall, 1981, enabled determination of the mechanisms of cold-hardiness and laboratory reared larvae were later used to test induced recoverability expected of freeze tolerant organisms. Larvae obtained from walnut husks in January,