Table 1. Effect of 2-deoxy-D-glucose on survival of UV-irradiated HeLa cells.

Irradiation time (sec)	2-DG conc. (mM)	Surviving fraction (%, mean ± SD)
—	—	96 ± 10
—	2.5	38 ± 12
30	—	90 ± 8
30	2.5	32 ± 10
60	—	88 ± 12
60	2.5	17 ± 9
90	—	76 ± 13
90	2.5	8 ± 6
120	—	40 ± 14
120	2.5	7 ± 5

Cell suspension in PBS ($2–3 \times 10^5$ cells ml^{-1}) was slowly stirred using a magnetic stirrer and a homogeneous suspension was exposed to UV light emitted by a low-pressure mercury vapour lamp (7G Philips–TUV 15 W) delivering the bulk of its radiation at 254 nm. Fluence rate measured by chemical actinometry[22] was 1.6 w m^{-2}.

presence of 2-DG (2.5 mM). Earlier investigations on peripheral blood leucocytes have shown that the repair of DNA and potentially lethal damage in yeast need a continuous flow of energy in the form of ATP[10,12].

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Effect of hypercholesterolaemia on mobility of erythrocyte membrane proteins

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Erythrocytes from rabbits fed a hypercholesterolaemic diet and human erythrocytes incubated in cholesterol-enriched plasma had increased cholesterol and a higher cholesterol-to-phospholipids ratio in the membrane. EPR studies revealed a decrease in ratio of signal due to weakly immobilized species to signal due to strongly immobilized species in the membrane, suggesting decreased membrane protein mobility in hypercholesterolaemic erythrocyte membranes.

THE normal protein composition of erythrocyte plasma membrane and the arrangement of the protein molecules in the membrane are crucial for membrane function. In hypercholesterolaemia the cholesterol content of plasma is increased, and in turn, the cholesterol to phospholipids (C/P) ratio is also high[1]. This has a direct influence on cholesterol transfer from plasma to erythrocytes, resulting in the accumulation of cholesterol in the erythrocyte membrane[2,3]. As binding of cholesterol to membrane constituents is weak, it can be drawn out of the membrane by decreasing the C/P ratio of the plasma, either by decreasing the cholesterol or increasing the phospholipid content of the plasma[4].

During the cholesterol accumulation process the structure of the membrane is slowly changed. At low concentrations spicules are formed on the membrane. With increase in cholesterol the erythrocytes acquire an echinocytic appearance[5,6] leading to a decrease in the haematocrit[7].

It has been observed[8] that increase in membrane cholesterol affects the availability of protein sulphhydryl groups at the surface. Cholesterol depletion results in decreased phosphorylation of the erythrocyte membrane protein spectrin[4]. In the membrane the specific and dynamic interactions between spectrin and other peripheral and integral proteins regulate their mobilities and associations[9].

Electron paramagnetic resonance (EPR) spectroscopy of membranes with incorporated nitroxide derivatives as spin labels[10] has been very useful in studies of membrane structure. The spin label 4-maleimido-(2,2,6,6-

tetramethylpiperidine-*N*-oxyl) (4-Mal-TEMPO) has been extensively used to modify erythrocyte membrane proteins. Changes in the EPR spectra of modified membranes are indicators of alterations in the motion and structural organization of membrane components, and have been used to examine a variety of membrane-associated processes[11]. Changes in membrane proteins[12] and lipids[13] are well established in hypercholesterolaemia. In this communication we report changes in cholesterol and phospholipid content and fluidity of membranes of (i) erythrocytes from rabbits fed a hypercholesterolaemic diet and (ii) human erythrocytes incubated *in vitro* in cholesterol-enriched plasma.

The hypercholesterolaemic diet of rabbits consisted of normal diet plus 0.5% cholesterol. Cholesterol-enriched human erythrocytes were obtained by incubating normal erythrocytes in cholesterol-enriched plasma (CEP) prepared as described earlier[14]. Erythrocyte lipids were extracted with a chloroform:methanol (2:1) mixture by the method of Folch *et al.*[15] Cholesterol[16] and phospholipids[17] of plasma and erythrocytes were determined as described earlier.

Membranes were spin-labelled[18] with 4-Mal-TEMPO (Sigma) in phosphate buffer (pH 8.0) at 4°C for 16 h in the absence of light, using the spin label in a 1.50 weight ratio to total membrane protein (estimated by the method of Lowry *et al.*[19]). The spin-labelled ghosts were extensively washed to remove excess unreacted 4-Mal-TEMPO until no nitroxide EPR signal could be detected in the supernatant. Spin-labelled erythrocyte membranes were taken in flat quartz cells for analysis. EPR spectra were obtained at room temperature using a Varian E4 spectrometer operating at 9 GHz and with $COORH_2$ modulation.

Cholesterol and phospholipid levels in plasma (Table 1) and erythrocyte membranes (Table 2) of normal and hypercholesterolaemic (hycholest) rabbits show increasing C/P ratio with increasing duration of feeding hypercholesterolaemic diet. Cholesterol and C/P ratio in human erythrocytes incubated in CEP were increased but there was no change in phospholipid level (Table 3).

Figure 1 shows the EPR spectra of free 4-Mal-

Table 1. Cholesterol, phospholipids and C/P ratio in plasma of normal and hypercholesterolaemic (hycholest) rabbits

	Cholesterol (mg %)	Phospholipids (mg %)	C/P ratio
Normal	71.12 ± 11.7	79.75 ± 7.75	0.89
Hycholest			
7 days	91.6 ± 14.7†	88.3 ± 11.3*	1.04
14 days	239.5 ± 16.2	144.9 ± 26.6	1.65
21 days	569.6 ± 43.0	262.6 ± 20.6	2.16
28 days	733.9 ± 49.9	314.3 ± 24.6	2.30
60 days	852.5 ± 41.6	368.8 ± 30.8	2.30
1 year	931.7 ± 50.5	398.5 ± 30.1	2.34

All values are mean ± SD.
*$P < 0.1$; †$P < 0.05$; after 14 days, $P < 0.001$ for all values.

Table 2. Cholesterol, phospholipids and C/P ratio in erythrocy of normal and hycholest rabbits

	Cholesterol (mg %)	Phospholipids (mg %)	C/P rat
Normal	48.6 ± 1.6	56.5 ± 6.8	0.86
Hycholest			
7 days	64.4 ± 8.0†	68.5 ± 10.1*	0.94
14 days	92.2 ± 12.3	79.0 ± 12.1	1.16
21 days	142.0 ± 13.3	98.5 ± 11.0	1.44
28 days	169.4 ± 13.9	101.6 ± 9.5	1.66
60 days	191.9 ± 26.1	117.0 ± 7.5	1.64
1 year	203.4 ± 42.9	121.7 ± 27.4	1.67

All values are mean ± SD.
*$P < 0.05$; †$P < 0.001$; after 14 days, $P < 0.001$ for all values.

Table 3. Cholesterol, phospholipids and C/P ratio in normal plasma, cholesterol-enriched plasma (CEP) and erythrocytes befc and after incubation in CEP

	Cholesterol (mg %)	Phospholipids (mg %)	C/P rati
Plasma			
Normal	155.5 ± 30.6	195.8 ± 30.7	0.79
CEP	265.1 ± 37.5*	190.5 ± 42.1	1.39
Erythrocytes			
Before incubation	99.28 ± 17.54	121.92 ± 25.99	0.81
After incubation	128.03 ± 20.23†	117.88 ± 10.27	1.08

All values are mean ± SD.
*$P < 0.001$; †$P < 0.05$.

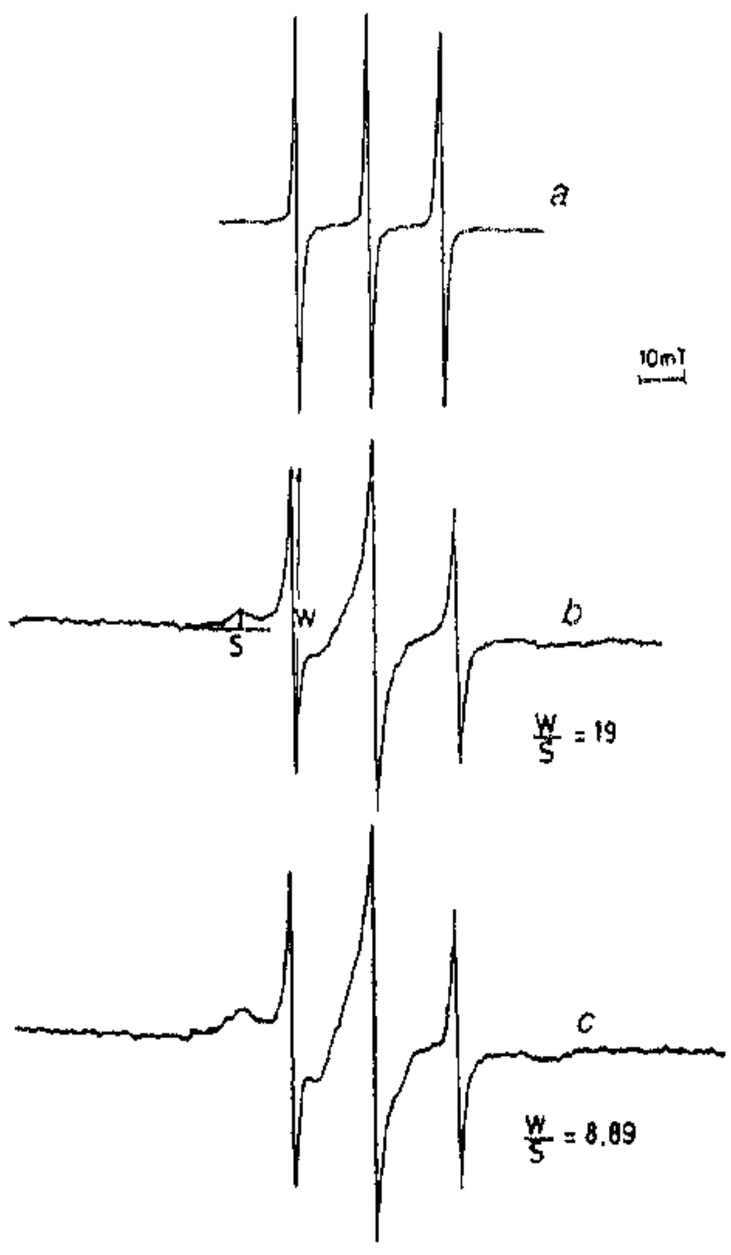

Figure 1. EPR spectra of (*a*) free 4-Mal-TEMPO (0.05 mM) i sodium phosphate buffer (pH 8.0), (*b*) spin-labelled normal rabb erythrocyte membranes, (*c*) spin-labelled hycholest rabbit erythrocyt membranes.

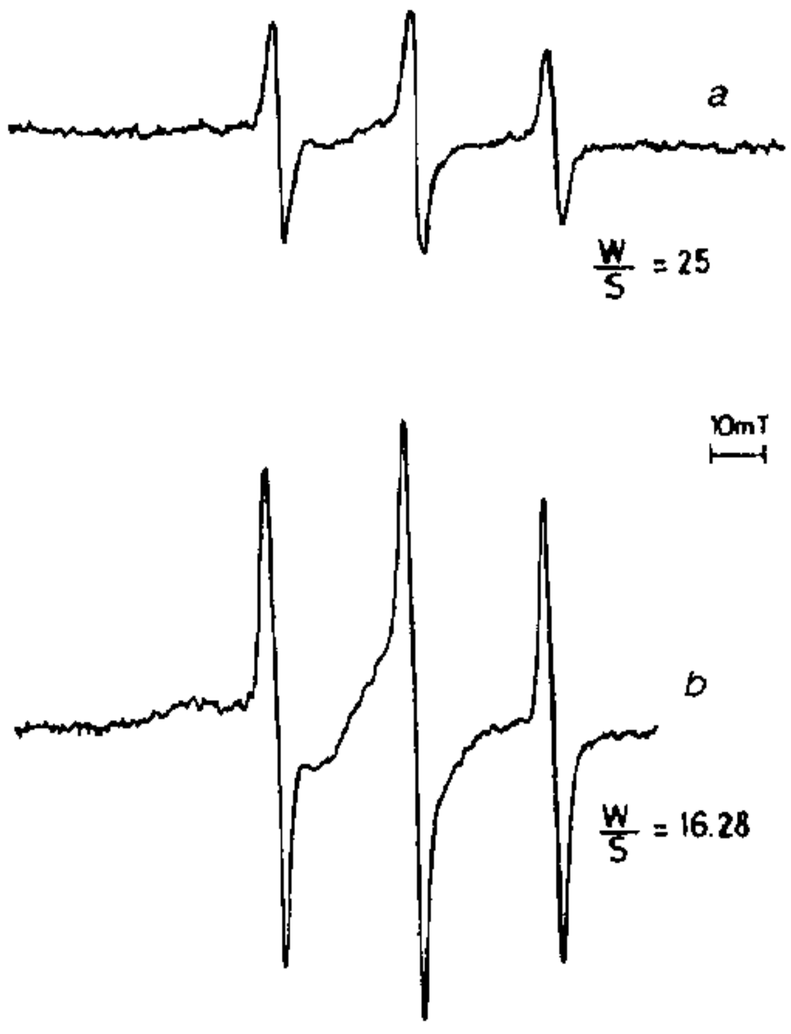

Figure 2. EPR spectra due to 4-Mal-TEMPO of (*a*) spin-labelled normal human erythrocyte membranes, (*b*) spin-labelled *in vitro* cholesterol-enriched human erythrocyte membranes.

TEMPO and spin-labelled normal and hycholest rabbit erythrocytes. The spectrum of the free spin label in aqueous solution contains a three-line pattern with a hyperfine coupling of about 15 G. When the spin label is attached to the protein part of the membrane it gives rise to a broad complex spectrum comprising two major components; one corresponds to weakly immobilized species (W) and another to strongly immobilized species (S). The W/S ratio is decreased significantly in hycholest membranes compared to that of controls. A similar result was obtained in the case of human erythrocytes incubated in CEP (Figure 2).

Erythrocyte membrane function is directly related to membrane composition, which can be altered by dietary regulation[20]. In the present study membrane composition was altered by feeding a hypercholesterolaemic diet to rabbits and by incubating normal human erythrocytes in cholesterol-enriched human plasma. Cholesterol content directly affects deformability, a parameter related to lipid and protein composition of the membrane[13,21]. Studies have shown that alterations in the protein cytoskeleton or membrane lipid composition of erythrocytes can affect membrane fluidity or microviscosity and, ultimately, the flow properties of erythrocytes[22,23].

According to the fluid mosaic model of membranes, membrane lipids allow free motion of proteins within the bilayer. This is altered in pathological conditions. EPR studies have suggested restricted motion of membrane proteins in erythrocytes from patients with hereditary spherocytosis[24]. In the present study, the W/S ratio, which affects mobility of proteins, was decreased in hypercholesterolaemia. This indicates that proteins are less mobile in cholesterol-enriched membranes. Under these conditions, the organization of proteins in the membrane may be altered, which results in abnormal morphology of erythrocytes.

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