Table 1. Effect of RU 486 on pregnancy in rabbits.

	Group I	Group II	Group III	Group IV
Dose	None (1% gum acacia)	6.4 mg/rabbit/day	32.0 mg/rabbit/day	160.0 mg/rabbit/day
Animals (*n*)	5	5	5	5
Total no. of implantations/implantation sites	26	25	24	19
Average no. of implantations/implantation sites per rabbit	5	5	5	5
Total no. of live births	23	Nil	Nil	Nil
Average no. of live births	5	Nil	Nil	Nil
Total no. of still births	Nil	Nil	Nil	Nil
Average no. of still births	Nil	Nil	Nil	Nil
Total no. of resorptions	3	25	24	19
Average no. of resorptions	1	5	5	4
Average foetal weight (g)	36.83	Nil	Nil	Nil
Average crown-rump length (cm)	5.05	Nil	Nil	Nil

though treated animals gained less weight compared to control animals. Maternal gain in weight in treated animals was dose-related. Whole foetus examination, Alizarin Red preparation for skeletal defect examination, and slicing method of Wilson for visceral defect examination revealed that (i) foetuses were not formed in any of the drug-treated groups, and only implantation sites were seen; and (ii) none of the foetuses of control group showed any gross or visceral defects. The results are summarized in Table 1.

RU 486 is a recently synthesized steroid with potent antiprogesterone properties, and presumably acts as a progesterone antagonist by blocking progesterone receptors. It is a proven effective medication for non-surgical termination of pregnancy. Local action of the compound on the endometrium quickly induces menstruation, though the exact dose regimen has not yet been established. In a clinical trial with women, the dose regimen employed (ranging from 100 mg/day × 7 days to 200 mg/day × 4 days or 400 mg/day × 4 days) terminated early pregnancy. It was found that lower dose for longer duration has a higher success rate than higher dose for shorter period[1].

The reason for lower effectiveness of a high-dose regimen is not known. If there is undue toxicity in early pregnancy, the embryo dies, is resorbed, and only the presence of the site of implantation is indicated[8]. For obvious reasons, this is termed resorption. In our study we observed implantation sites in the uteri of drug-treated rabbits. However, administration of RU 486 in low, high and toxic doses (6.4 mg, 32.0 mg and 160.0 mg/rabbit respectively) during the period of organogenesis produced 100% resorptions in all the animals. We therefore conclude that RU 486 is an effective embryotoxic agent at all the doses used in the present study.

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Effect of 2-deoxy-D-glucose on HeLa cells

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The effect of 2-deoxy-D-glucose (2-DG), an inhibitor of glycolysis and glucose transport, on growth and survival of unirradiated and UV-irradiated HeLa cells was investigated. Addition of 5 mM 2-DG to cultures resulted in reduction of the number of viable cells to 18.5% of that of control. 2-DG (2.5 mM) also increased cell mortality in UV-irradiated cultures.

2-DEOXY-D-GLUCOSE (2-DG) is a known glucose anti-metabolite and an inhibitor of glycolysis[1,2]. 2-DG can act in a number of ways, the chief route of action being in its capacity to inhibit competitively both phosphorylation (hexokinase) and transport of glucose[3-6]. Catabolism of cellular nucleotides (chiefly adenosine) to nucleosides and bases[7] is another route of action of destabilizing the cellular energy system[8-10].

Further, 2-DG has been shown to inhibit repair of

radiation-induced cellular and DNA damage in respiratory-deficient yeast cells[11,12], as well as mammalian cancer cells[10,13,14]. On the other hand, 2-DG has also been shown to have no effect on or to enhance repair of potentially lethal or sublethal cellular or DNA radiation damage in normal cells[13,15-18] and wild-type yeast cells[19].

These observations suggest that 2-DG could differentially inhibit repair of radiation damage in hypoxic cancer cells, but, under certain conditions, enhance repair in normal cells, and therefore, possibly, be used for optimizing radiotherapy of cancer.

The strain of HeLa cells used in the present study originated in the National Institute of Virology, Pune. Cells were grown in minimum essential medium (MEM; from Bios, Bombay) supplemented with 10% calf serum, containing antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin and 25 μg/ml kanamycin), and buffered with 20 mM Hepes buffer (Serva, Heidelberg, FRG) to maintain pH at 7.4. Stock cultures were grown at 37°C in 150 ml glass culture bottles containing 20 ml of medium. Culture medium was changed every 48 h. Cell density was determined at various times in cell suspensions obtained after trypsinization (0.1% trypsin, 1 min at 37°C) by counting the cells in a haemocytometer. Viability of cells was determined by the dye exclusion test. For experiments on post-irradiation survival and proliferation kinetics, cells were grown on cover slips in Leighton tubes containing 2 ml of growth medium. Medium was discarded from a fortyeight-hour-old HeLa cell culture. The cells were washed with phosphate-buffered saline (PBS), trypsinized, and then incubated at 37°C for 1 min. Cells were again washed with PBS and resuspended in the same and counted. Three-millilitre aliquots of cell suspension were placed in marked petri dishes and irradiated with UV light (254 nm) for different times. Controls were also set up for each experiment. Immediately after UV irradiation, the cell suspensions were pipetted out and centrifuged to pellet the cells. The supernatant PBS was decanted and 4 ml of growth medium containing 2.5 mM 2-DG (Serva) was added to each tube. The cell suspensions were then transferred to appropriately marked Leighton tubes. After incubation at 37°C for 48 h the cells were again trypsinized, and washed and resuspended in 1.0 ml PBS. Viable cells were counted in a haemocytometer by the dye exclusion test. Glucose[20] and lactate[21] were also estimated.

The number of viable cells in 48-hour-old cultures in medium containing 2-DG was only 36.5% of that in control cultures. In medium containing 5 mM 2-DG, it was 18.5%, and only 13% in medium containing 10 mM 2-DG. Multiplication of cells could be restored even after 48 h simply by removing the inhibitor, washing the cells, and adding complete growth medium. However, the effect was irreversible after 72 h of incubation.

Figure 1 shows the effect of 2-DG on glucose utilization and aerobic glycolysis (lactate production) in HeLa cells. These results are similar to data obtained earlier in animal tumours[16].

Table 1 shows the effect of 2-DG on survival of UV-irradiated HeLa cells in the presence of 2.5 mM 2-DG. 2-DG was present only for 2 h immediately after UV irradiation. It can be seen that the decrease in viable cell number due to UV irradiation is enhanced in the

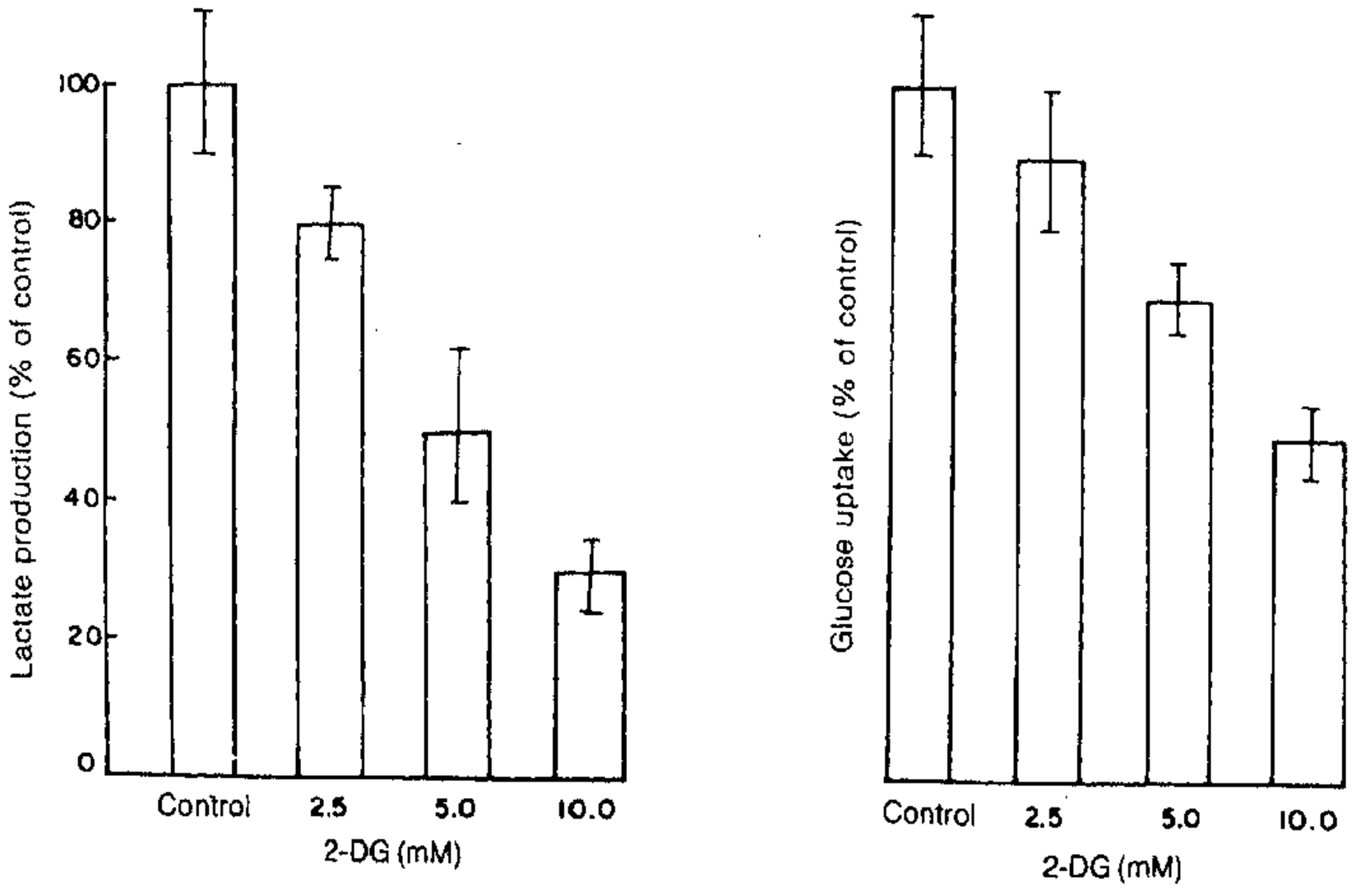

Figure 1. Effect of different concentrations of 2-deoxy-D-glucose on energy metabolism in HeLa cells.

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-