Effect of Deuterium Oxide on Growth of HeLa, L and L-5178Y Cells.*† (26148)

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Many studies have been carried out on the effects of deuterium oxide on growth of plants, bacteria, protozoa and animals(1). In general, these studies have shown that deuterium in high concentrations is toxic in some degree to all living organisms. As the organism under study becomes more highly organized deuterium becomes more toxic, thus algae can grow in 99% D_2O(2), while mice cannot survive 40% deuteration(3). The toxic effect of high concentrations of deuterium oxide on cells grown in tissue culture was first observed by Fischer, working with chick fibroblasts, a mouse carcinoma and Rous sarcoma(4). Cagianut(5) commented on certain aspects of the cytopathology of tissue cultures of rabbit connective tissue and recently Siegel *et al.*(6) have studied the cytopathology of HeLa cells grown in D_2O. The common observations have been cell clumping, cell enlargement, multiple nuclei, nuclear pyknosis and vacuolation. Some of these characteristics have also been seen in D_2O-grown *E. coli*(7). This report deals with effects of D_2O on growth and cytological characteristics of 3 lines of tissue culture cells.

Results and discussion. Three cell lines of diverse origins were used: HeLa, a stable epithelial line of human origin; L cells, a murine fibroblast strain; and L-5178Y, a stable cell line derived from a spontaneously occurring murine leukemia of thymic origin. HeLa and L cells are usually grown as a monolayer on the surface of glass containers; they generally exhibit mass doubling times of 24-48 hours. L-5178Y is a round cell which does not adhere to glass and therefore can be grown in suspension as either a stationary or agitated culture(8). It is unique in that it shows an 11.5-hour generation time at 36°C during logarithmic growth.

TABLE I. Effect of D_2O on Yield of HeLa and L Cells.

% D_2O in growth medium	Total yield of cells* HeLa	L
0	100	100
10	87	85
20	64	54
30	49	34
40	23	16
50	13	4

* As % of control.

The studies with HeLa and L cells were carried out in Leighton tubes. Freshly trypsinized cells (150,000) were planted per tube in 1 ml of Eagle's basal medium, 10% in calf serum, containing varying amounts of heavy water. The medium in each tube was changed on alternate days. After 8-10 days at 36°C the cells were harvested with trypsin or trypsin-versene and cell yields determined (Table I). Cell counts were determined by means of hemocytometers. Each count reported is the mean of 4 replicate determinations and shows a statistical variation of ± 10%. It can be seen that 10% heavy water has little effect on growth rate, whereas 40 and 50% heavy water is toxic. The final number of cells recovered at these D_2O concentrations (40 and 50%) was less than the original inoculum.

The influence of D_2O on mass doubling time of logarithmically growing L-5178Y is shown in Table II. With this rapidly growing cell, an effect can be seen even at 5% D_2O. At 30% D_2O, the mass doubling time is almost twice the normal value. At all levels of D_2O below 40% the cells grew logarithmically for at least several weeks. The value found in 50% D_2O can be determined only

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TABLE II. Effect of D_2O on Mass Doubling Time, Wet Weight and Dry Weight of L-5178Y Cells.

D_2O in growth medium (%)	Mass doubling time (hr)	Wet wt	Dry wt
		(mg/10^6 cells)	
0	11.5	.71	.12
5	12.3	.75	.12
10	13.0	1.20	.15
20	14.2	1.20	.18
30	19.0	1.50	.15
40	29.0	1.50	.15
50	56.0	—	

over the first 96 hours after exposure of the cells to this medium. During this time the cells grew; then cell counts began to decrease and the culture died. This leukemia was grown in the medium devised by Fischer(8).

A more detailed investigation was made of the effect of D_2O on L-5178Y and HeLa cells. In Table II are shown wet weight and dry weight of L-5178Y cells raised in media of varying D_2O concentration. There is a distinct increase in water content of the cells as D_2O concentration in the growth medium is increased. There is also a slight increase in dry weight of the cells.

HeLa cells were exposed to a medium containing 20% heavy water for varying lengths of time, and the wet weight and dry weight of the cells determined (Table III). Again we see a slight increase in both dry weight and water content of the cells. The composition of these deuterated cells with respect to proteins, nucleic acids, polysaccharides and lipids is now under investigation.

Cytological observations. The morphological and cytochemical changes induced by D_2O in 2 cellular systems *in vitro,* HeLa and L cells, are essentially similar. Briefly, in both cell types one can observe a progressive increase in number of large polynucleated cells paralleling the increase in concentration of D_2O in the medium. This effect is more evident with the HeLa line, in which multinucleated cells are present in only 1-2% of the normal cell population; thus even in 10% D_2O the number of multinucleated cells is increased, and at 40% D_2O, between 30-35% of the cell population is made up of large cells with 2-3 nuclei, and occasionally more, with an enormous cytoplasm (Fig. 1-4).

TABLE III. Effect of 20% D_2O on Wet and Dry Weight of HeLa Cells.

Time of exposure (days)	Control (H_2O)*		D_2O*	
	Wet wt	Dry wt	Wet wt	Dry wt
3	5.7	.65	5.2	.70
6	5.5	.70	6.5	.80

* Values are mg/10^6 cells. Weights given are avg of 4 determinations and show a variation of less than 2%. They are subject to the statistical limitations of the cell counting method.

The cytoplasm appears as an amorphous protoplasmic mass, and even with phase contrast, very few mitochondria can be discerned; the Golgi areas seem to be hypertrophic. The nuclei are slightly enlarged; no particular variations in size and number of the nucleoli has been observed. The general cytological characteristics of the deuterated cells are, then, very similar to those of the giant cells produced by irradiation(9). In the case of L cells, enlarged and polynucleated forms are also present, but not to the striking degree observed with HeLa cells; in addition, L cells in 20% D_2O lose their characteristic cytoplasmic filament, the cell border becoming poorly defined. No significant changes in regard to DNA and RNA were demonstrable by Feulgen and pyronin staining. In only one experiment with L cells was there a striking loss of cytoplasmic RNA, unaccompanied by changes in the nucleolar RNA. Lipid stains (Sudan black and Nile blue sulphate) have given equivocal results; in general, we observed a moderate increase of sudanophilic material and a shift in the morphology of this material in the sense that the small, uniform granules—lipochondria—which are present in the normal cells, decrease, while large, irregular masses of sudanophilic material increase. This increase has not been uniform in various experiments and seems to be more evident with L cells. In one HeLa experiment, there was a suggestion of an increase in neutral fat, as demonstrated by Nile blue sulphate. It is probable that the extent of lipid changes is related to the general condition of the cells at time of deuteration, and possibly to the length of time they are exposed to heavy water.

Summary. The effect of D_2O on growth of 3 stable mammalian cell lines (HeLa, L

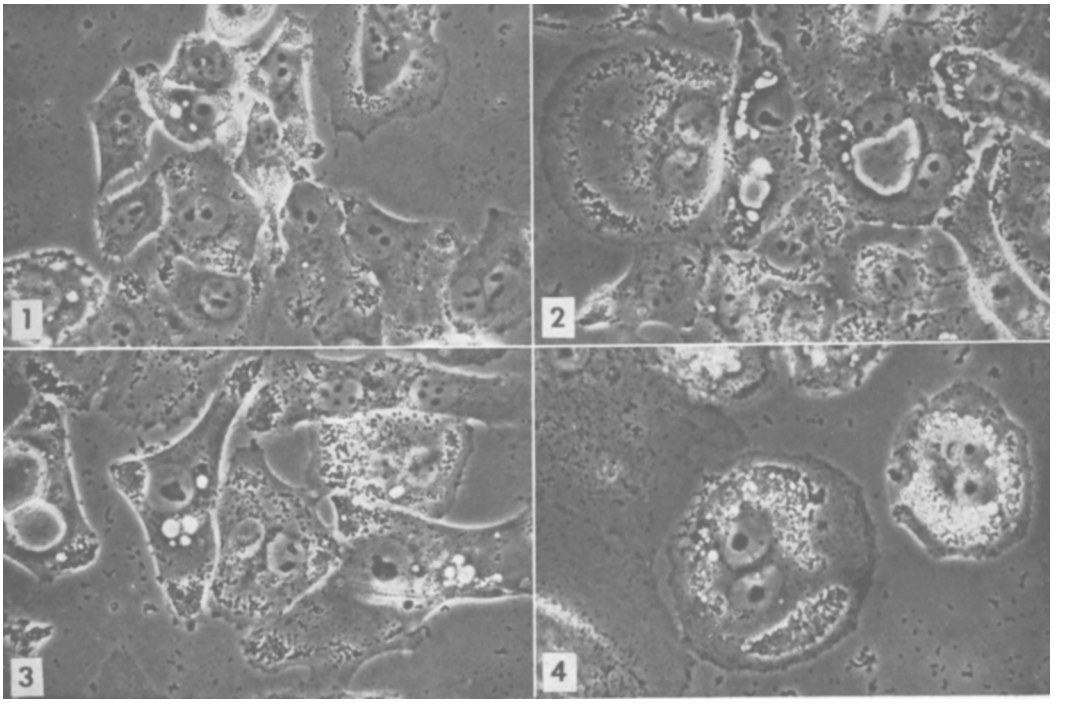

FIG. 1. HeLa cells in H_2O-containing medium. Cytoplasmic and nuclear characteristics are evident. Phase contrast × 293.

FIG. 2. HeLa cells in 20% D_2O-containing medium. This field is occupied by a large binucleated cell and by mononucleated cells which show an increased size. In addition, conglomerates of refractile granules (lipochondria) are visible at periphery of cytoplasm. Phase contrast × 293.

FIG. 3. HeLa cells in 30% D_2O-containing medium. All cells are enlarged and several are binucleated. Several cells have large cytoplasmic vacuoli which are sudanophilic in stained preparations. Phase contrast × 293.

FIG. 4. HeLa cells in 40% D_2O-containing medium. There has been a decrease in cell number and all residual cells show a higher degree of the changes illustrated in previous pictures. In this field 2 enlarged binucleated cells are seen together with a large portion of cytoplasm of another giant cell. Phase contrast × 293.

and L-5178Y) has been investigated. As D_2O concentration was increased all cells showed increased water content and dry weight and a slower growth rate. Cytological investigations showed an increase in number of multinucleated cells and a moderate increase in sudanophilic material. No significant changes in nucleic acid content were demonstrable with the staining technics used.

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