Intracellular Fluoride in Cultured Mammalian Cells (36115)

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Most investigations of the sensitivity of cultured mammalian cells to fluoride have been concerned with a determination of the levels of fluoride which inhibit growth. While some studies (1, 2) have suggested that the growth of mammalian cells can be inhibited by from 0.045 to 2 ppm F, most reports (3–8) have indicated that a minimum of 5–15 ppm F (0.3–0.8 m*M*) is required to decrease the rate of cell division or accumulation of cell protein. At higher levels of fluoride, there appears to be a sigmoidal dose–response relationship with slight inhibition at 10 to 20 ppm and complete inhibition of growth at 40 to 50 ppm (6, 7). Although it has been observed that cellular ATP levels are decreased (9), and glucose uptake per milligram of cellular protein is increased (8, 9) in fluoride-treated cells, the mechanism by which fluoride inhibits growth is not understood. To further characterize the toxicity of fluoride in mammalian cell cultures, the distribution of fluoride between cells and medium was determined. A comparison of intracellular fluoride concentrations with the concentrations of fluoride reported (10) to inhibit various enzymes *in vitro* may allow a prediction as to enzyme inhibitions of physiological significance.

Methods. Suspension cultures of HeLa CCL 2 cells and L cells (mouse fibroblasts) with generation times of 36 and 19 hr, respectively, were utilized for these experiments. The L cells were grown in medium B of Medappa *et al.* (11) and the HeLa cells were grown in the medium described by Carlson and Suttie (9) modified by the substitution of a Ca–Mg-free salt mixture (12) and the addition of 0.1% Pluronic F-68 detergent. The cells were resuspended in fresh medium at a cellular concentration of 4×10^5 cells/ml for an equilibration period of 1 hr before measuring fluoride distribution. A mixture of carrier-free ^{18}F prepared by neutron activation (13) and NaF in a total volume of 3 ml was added to 200 ml of cell suspension in a 500 ml Erlenmeyer flask, and the cells were incubated for 1 or 2 hr at 37° on a rotatory shaker. The cell suspensions were then centrifuged at 500*g* for 1–2 min; and the cell pellets were resuspended in 10 ml of supernatant. After the addition of 10 μCi of ^{14}C-inulin, 2.5 ml aliquots of cell suspension were injected into specially designed glass centrifuge tubes (14) having a reservoir of 2.5 ml capacity connected with a capillary 6 cm long and 1 mm in diameter and centrifuged at 2000*g* for 20 min at 4°. The cell pellets and supernatant medium recovered from centrifugation were analyzed for ^{18}F and ^{14}C-inulin according to standard procedures. Since inulin cannot penetrate mammalian cells, the ^{14}C in the cell pellets was used to calculate the amount of entrapped medium. Intracellular fluoride concentrations were calculated from the amounts of ^{18}F in cell pellets and entrapped medium, the specific activity of ^{18}F in the medium, and the amount of intracellular water in pellets. Intracellular water values were calculated from the wet and dry weights of cell pellets and the volume of entrapped medium. Cellular protein was determined by a modification (15) of the Lowry method.

Results and Discussion. The effect of medium fluoride concentration on the growth rate of L cells is illustrated in Fig. 1. There was no detectable effect of 10 ppm F on the growth of the cells, and a very pronounced

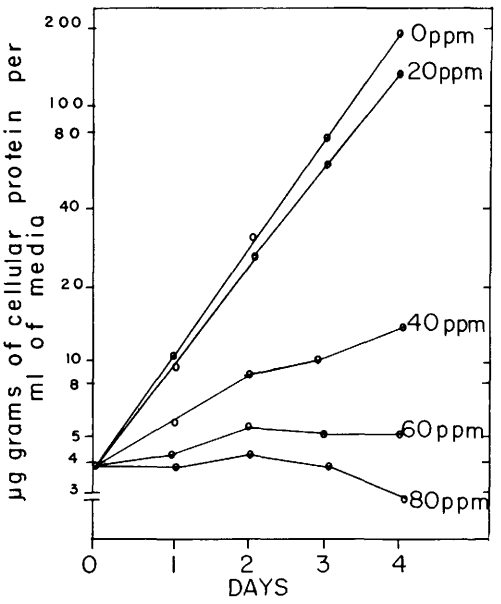

FIG. 1. Effect of fluoride concentration on L cell growth.

effect at 50 ppm F, which was the other level at which fluoride distribution was determined. The HeLa cell line used had a generation time of about 36 hr, and sensitivity to fluoride was similar to that shown for L cells. The percentage of the wet cell weight, which was water, ranged from 77 to 80% in both HeLa cells and L cells incubated with no fluoride, and this value was not altered when the cells were treated with 10 or 50 ppm F.

The data on intracellular fluoride concentrations are shown in Table I. The distribution ratio, F_{in}/F_{out}, did not appear to be greatly influenced by the differences in extracellular fluoride concentration, duration of incubation, or cell line. The average ratio of distribution for all treatments was 0.37. The data would suggest, however, that the distribution ratio was somewhat higher at the lower fluoride concentration. There may also have been a slight increase in the amount of fluoride penetrating the HeLa cells at the higher fluoride concentration. These cells are also slightly more sensitive to fluoride (7).

From the results shown in Table I, it appears that the intracellular fluoride concentrations effective in inhibiting, but not completely preventing the growth of these cells range from 4 to 20 ppm (0.2–1.0 m*M*). This range of fluoride concentrations has been demonstrated *in vitro* to significantly inhibit glutamine synthetase, enolase, cytochrome oxidase, and a number of esterases and lipases (10). The physiological significance of inhibition of these enzymes on growth inhibition in mammalian cell culture is currently being investigated.

Summary. The intracellular concentration of fluoride in HeLa cells and L cells incubated in 10 or 50 ppm F was determined. For both cell types, the distribution ratio F_{in}/F_{out} ranged from 0.3 to 0.4 and was not greatly influenced by the length of incubation or medium fluoride concentration.

TABLE I. Intracellular Fluoride Concentrations.

F (ppm) in incubation media	Incubation period (min)	L Cells Expt. I (ppm F)	L Cells Expt. II (ppm F)	HeLa cells (ppm F)
10	60	4.24 ± 0.05 (4)[a]	4.10 ± 0.27 (4)	3.54 ± 0.30 (4)
	120	4.42 ± 0.20 (4)	3.85 ± 0.10 (3)	4.46 ± 0.53 (3)
50	60	14.08 ± 0.40 (4)	16.23 ± 0.80 (2)	20.15 ± 0.90 (4)
	120	13.84 ± 0.25 (4)	14.62 ± 0.90 (3)	20.92 ± 0.37 (4)

[a] The standard error of the mean and the number of determinations per average are given.

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