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Comparison of Effects of Deuterium Oxide and X-Ray Irradiation on Multiplication of Poliovirus.* (27960)

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In studies on the effects of deuterium oxide on various biological systems we have observed that the multiplication of poliomyelitis virus is enhanced in tissue culture systems in which the medium contains 20-40% of D_2O(1,2). This effect was observed with several strains of poliovirus–Mahoney, MEF-1, and CHAT (an attenuated type 1 virus)–and with various host cells, among them HeLa and stable and primary lines of monkey kidney cells. Using KB cells and poliovirus I, Lwoff and Lwoff have made similar observations(3,4). In speculations concerning the mechanism of action of D_2O(5) we noted the possibility that the D_2O effect may be similar to the actions of X-rays since both deuteration(2,6) and X-ray irradiation(7) result in formation of giant cells and both treatments result in increased virus yield when these cells are infected(1,8). Since CHAT, a virus which normally does not replicate at incubation temperatures as high as 40°C, will reproduce at 40°C in deuterated medium(1,5,9), it was of special interest to ascertain whether this virus would also replicate at 40°C in X-irradiated cells. In preliminary experiments (5) we studied this possibility using only one level of irradiation (300 r). This communication covers a broader investigation into this phenomenon.

Materials and methods. Monolayer cultures were prepared from primary trypsinized monkey kidneys by technics previously outlined(9). Two days after seeding the Petri dishes, the developing monolayers were treated with medium containing D_2O, with X-ray irradiation, or with both. To yield a growth medium containing 25% D_2O, half of the standard growth medium was removed and replaced with a 50% D_2O medium that has been previously described(9). The X-ray irradiation of monolayers was done in covered Petri dishes with the medium present. After the irradiation the pH of the medium, which had risen markedly, was adjusted to pH 7.4 by addition of a few drops of 0.1 N HCl. Control monolayers were treated in the same way except that D_2O or X-ray irradiation was omitted. Five days after addition of medium containing D_2O or the X-ray irradiation treatment, the monolayers were infected and overlaid with agar-overlay medium in a manner previously described(9).

X-ray irradiation was done by using a Keleket X-ray tube operating at 200 KV and 20 m.a. at a distance of 30 cm. No filters were used. The rate of irradiation was monitored and dosage was varied by altering the time of exposure to X-ray.

The technics for determining cell number and volume were as follows: the cell mono-

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TABLE I. Effect of D_2O* and X-ray Irradiation upon Plaque Formation by CHAT Virus in MK Monolayers at 37°C and 40°C.

Exp. No.	Treatment of monolayer	No. of plaques 37°C	40°C
1	Control	34	0
	D_2O	6	25
	X-ray 470r	49	0
	950r	28	0
	1400r	9	0
	1900r	8	0
2	Control	120	0
	D_2O	23	82
	X-ray 1425r	96	0
	2850r	87	0
3	Control	10	0
	D_2O	22	30
	X-ray 250r	46	0
	500r	56	0
	750r	46	0
	1000r	48	0
4	Control	96	0
	X-ray 250r	123	0
	500r	102	0
	1000r	144	0
	1500r	144	0

* 25% D_2O in cell growth medium and 50% D_2O in overlay medium.

layers were trypsinized 10 days after application of deuterium or X-ray irradiation. Cells were counted in a hemacytometer. The cell volume was found by centrifugation of a known number of cells in 10 ml centrifuge tubes graduated from 0 to 0.1 ml in 0.005 ml dimensions.

Results and discussion. Initially, it was important to ascertain whether there was any level of irradiation at which MK cell monolayers would support the growth of CHAT virus at 40°C. Table I presents a comparison of deuteration and X-ray irradiation on plaque formation by CHAT virus at 37°C and 40°C. In all experiments, there was plaque formation by CHAT virus in the X-rayed cells at 37°C, but at 40°C only the deuterated cells yielded plaques. Since earlier experiments had shown that Mahoney virus could replicate at 40°C, the irradiation experiments were repeated comparing Mahoney with CHAT virus in irradiated cells at 37°C and 40°C (Table II). Most noteworthy are the observations that irradiated cells yield plaque formation by Mahoney virus and that addition of deuterium oxide to X-rayed cells results in plaque formation by CHAT virus at 40°C. These data show that the X-irradiated cells are competent at 40°C but are incapable of providing a milieu suitable for growth of CHAT virus at that temperature. A change wrought by addition of deuterium oxide to the X-rayed cells renders them an effective substrate for the growth of CHAT virus at 40°C. Clearly, the effects of X-ray irradiation and deuteration on ability of the cells to support growth of virus are different.

Subsequent experiments were carried out to gauge the effects of the 2 treatments on virus yield per infected cell and on cell size and volume. At 37°C X-ray irradiated cells provide a better substrate for the growth of CHAT virus than do cells treated with D_2O, but at 40°C only deuterated cells support the growth of CHAT virus (Table III). At 37°C the yield per X-ray irradiated cell is approximately 2 times the yield per deuterated cell and 3 times the yield in control cells. These results correlate with the finding that plaque sizes at 37°C are increased in X-ray irradiated and deuterated cells. The question of the number and type of cells available (*i.e.*, substrate) in the 2 treatments then arises. In a comparison of the number of cells in monolayers treated with D_2O or X-ray at the 2 temperatures, it is seen that at 37°C, when cells are irradiated with 2000r, the number of cells per monolayer is reduced to about half the number of cells in monolayers treated with D_2O and to one-

TABLE II. Plaque Formation of Mahoney and CHAT Viruses on Irradiated Cells (MK) at 37°C and 40°C.

Exp No.	Virus	Treatment of monolayer	No. of plaques 37°C	40°C
1	Mahoney	Control	79	90
		X-ray 500r	75	62
		1000r	72	74
		1500r	63	85
		2000r	65	69
2	CHAT	Control	>320	0
		X-ray 500r	>320	0
		500r + D_2O (50%)	>320	>320
		2000r	>320	0
		2000r + D_2O (50%)	>320	>320

TABLE III. Growth of CHAT Poliovirus in D_2O-treated and X-ray Irradiated Cells at 37°C and 40°C (MK Monolayers).

Exp No.	Treatment of monolayer	No. cells per monolayer ($\times 10^6$)	Virus yield per monolayer 37°C ($\times 10^8$)	Virus yield per monolayer 40°C ($\times 10^6$)	Estimated burst size 37°C	Estimated burst size 40°C
1	Control	3.47	2.5	—	75	0
	D_2O (25%)	1.54	1.8	3.4	113	2.2
	X-ray (2000r)	.92	2.2	—	237	0
2	Control	3.08	5.8	—	156	0
	D_2O (25%)	1.95	4.6	6.1	238	3.1
	X-ray (2000r)	1.04	4.2	—	408	0

quarter to one-third the number of control cells (Table IV).

Levine(10) has observed that a type 3 poliovirus (Leon strain) when grown in primary monkey kidney cells X-irradiated at a level of 1500-2000r will give a 5-fold increase in plaque-forming units when compared with non-irradiated cells. The irradiated cells are 4-6 times as large as the unirradiated cells, based on cell volume measurements carried out 6 to 7 days after plating. We have measured the volume of deuterated (25% D_2O) and X-irradiated (2000r) cells 8 days after treatment with results shown in Table V. Deuteration increases the size by 33% and X-irradiation by 66%.

The mechanism by which deuterium oxide treatment exerts its effect is still not clear. The increased size of irradiated cells has been shown to be due to continued synthesis of large molecules (growth) in cells which can no longer divide(11). Whether there are differences in the type of substrate for viral growth provided by X-irradiated and deuterium oxide treated cells is under investigation.

TABLE IV. Effect of D_2O and X-ray Irradiation on Average Number of Cells in Monkey Kidney Monolayers.

Exp No.	Treatment	Avg No. of cells* (%) 37°C	Avg No. of cells* (%) 40°C
1	Control	100	95.0
	D_2O (25%)	86.3	79.6
	X-ray 500r	78.1	70.6
	2000r	36.8	32.9
2	Control	100	85.7
	D_2O (25%)	43.9	36.1
	X-ray 500r	54.4	42.6
	2000r	27.2	20.9
3	Control	100	
	D_2O (25%)	40.4	
	X-ray 2000r	26.5	
4	Control	100	
	D_2O (25%)	63.3	
	X-ray 2000r	33.8	

* Expressed as % of control cells at 37°C.

TABLE V. Volumes of Monkey Kidney Cells 8 Days After Treatment with D_2O (25%) or X-ray (2000r).

Exp No.	Treatment	Total cells ($\times 10^7$)	Vol. (ml)	Avg cell vol. (ml $\times 10^{-8}$)
1	Control	1.25	.073	.58
	D_2O	.66	.051	.77
	X-ray	.20	.037	1.85
2	Control	1.09	.058	.53
	D_2O	.34	.030	.88
	X-ray	.24	.041	1.71

Summary. An attenuated strain of poliomyelitis virus (CHAT) will not grow in monkey kidney cells at 40°C. When deuterium oxide (25-40%) is present in the medium, replication of CHAT virus will take place at 40°C. Since both deuterium oxide treatment and irradiation with X-rays yield "giant" cells, the 2 treatments have been compared for their ability to support the growth of CHAT poliovirus at 40°C. At several levels of X-irradiation, monkey kidney cells will not support the growth of CHAT virus at 40°C. When D_2O is added to the medium of the X-irradiated cells at 40°C, replication of CHAT virus is observed. The effect is not due to cell size or number.

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Mechanism of Aminonucleoside-Induced Nephrosis in the Rat. III. Kidney Mitochondrial Phosphorylation and Dephosphorylation Activity.* (27961)

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During speculation on the chemical and physical nature of metabolic alterations which might explain the onset and development of the nephrotic syndrome in the rat treated with aminonucleoside, 6-dimethylamino-9-(3′ - amino - 3′ - deoxy - β - D - ribofuranosyl)-purine, the possibility was considered that some of the nephrotogenic action of the aminonucleoside might be due to interference with kidney mitochondrial phosphorylation and/or dephosphorylation reactions.

Results of exploratory studies of phosphorylation associated with oxidation of succinate might be considered as indirectly supporting the view that ATP formation is inhibited since both oxygen uptake and disappearance of inorganic orthophosphate are significantly reduced in kidney mitochondria of aminonucleoside-nephrotic rats(1). Several other considerations, however, suggest that this effect may be secondary in origin. Fisher *et al.*(2-3), working with kidney slices of aminonucleoside-nephrotic rats, report not only a significant depletion of histochemically demonstrable succinic dehydrogenase and cytochrome oxidase in the proximal convoluted tubules and ascending limbs of Henle, but also an apparent direct relationship between degree of depletion of the tubular enzymes and degree of proteinuria. This finding, together with the failure of aminonucleoside added directly to normal rat kidney slices to alter oxygen consumption, led these investigators to conclude that the alterations in enzymatic activity might be due to the proteinuria of the disease.

In addition to considering this possible explanation for our findings, the possibility must be considered that the observed reduction in disappearance of inorganic phosphate is due to a leak of phosphate from the organic to the inorganic orthophosphate pool. Thus dephosphorylation of adenosine triphosphate (ATP) and glucose-6-phosphate (G-6-P), both of which are formed in the oxidative phosphorylation reaction, and/or adenosine diphosphate (ADP), added as a phosphate acceptor, would either singularly or in combination effect an apparent reduction in disappearance of orthophosphate.

Studies were undertaken to determine the time-course of development of the proteinuria of the rat treated with aminonucleoside in relation to induction of the indicated alterations in kidney mitochondria(1); to evaluate the possibility of interference of dephosphorylation of ATP, G-6-P, and ADP with

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