Effects of Deuterium Oxide upon Plaque Formation and Replication of Simian Virus$_{40}$.* (28428)

RICHARD I. CARP, IVAN CHUDNOW, HILARY KOPROWSKI, and DAVID KRITCHEVSKY
Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

The substitution of deuterium oxide (D_2O) for water in the medium used for maintenance of tissue culture cells infected with poliomyelitis virus has a pronounced effect upon virus replication in these cells(1-4). The effects of D_2O in infected cultures kept at 37°C include an increase in virus yield per cell as well as a slight increase in average plaque diameter(5). Even more dramatic effects are observed when D_2O containing medium is used for cultures incubated at 40°C, a temperature at which the reproductive capacity of certain strains of poliovirus is markedly inhibited in conventional medium. When the medium is made up to contain 25-50% D_2O, virus replication is restored to a considerable extent(1-5).

The effect of D_2O upon replication of other viruses is under study in our laboratory. Results obtained in studies with Simian Virus$_{40}$ (SV_{40})(6) are the subject of this report. SV_{40} displays several interesting properties: namely, tumor formation in hamsters(7) and transformation of human cells (8,9).

Materials and methods. SV_{40} and SV_{40}-immune serum used in these experiments was generously supplied by Dr. Bernice Eddy. Grivet monkey kidney (GMK) was used in all experiments for growth of virus. The techniques for maintenance, infection and overlay of the cells were essentially those used in earlier experiments involving poliovirus and Rhesus monkey kidney cells(2) with the following exceptions: (a) The virus was adsorbed for 90-120 minutes; (b) In the nutrient overlay medium used for plaque assay, the final concentration of Noble agar was 0.085%; (c) Since the monolayers used for plaque assay were maintained for 14 days, a second nutrient overlay (2.5 ml) was added on the 7th day of incubation and the overlay containing Neutral Red was added on the 10th day. Each nutrient overlay was made up in aqueous medium (control) or in medium containing 50% D_2O. Plaques were counted and observed from the 11th to the 13th day.

In tube titration experiments, GMK tissue culture in tubes from which the maintenance medium had been removed was exposed to 0.2 ml of Hanks' BSS containing $10^{5.7}$ $\log_{10}$ plaque forming units (PFU) of SV_{40}. After 90 minutes the virus inoculum was removed and the cells were washed 3 times with 3.6 ml of Hanks' BSS. Following this, either conventional Eagle's in Earle's medium or the same medium containing 25% D_2O was added to the cells.

Results and discussion. The results of an initial series of experiments are summarized in Table I. At 37°C (Exp. 1-6) the titer of virus (as PFU/1 ml) was greater in the monolayers maintained under H_2O medium overlay than in D_2O medium. This difference was also observed in monolayers maintained at 40°C. Excluding those plates in which generalized degeneration of monolayers was observed (possibly due to long exposure to D_2O), the average titers of SV_{40} (in $\log_{10}$ PFU/1 ml) for monolayers of GMK maintained at 37°C under aqueous or 50% D_2O medium were 6.8 ± 0.2 (standard deviation) *vs* 5.9 ± 0.3 at 11 days (3 experiments), 6.9 ± 0.2 *vs* 5.0 ± 1.1 at 12 days (4 experiments) and 7.3 ± 0.02 *vs* 6.4 ± 0.2 at 13 days (5 experiments). At both 37°C and 40°C plaques appearing in the plates kept under D_2O-containing medium were smaller than those seen in control plates. Plaques picked from plates in which SV_{40} was grown under either medium were neutralized by SV_{40}-immune antiserum.

The relative yield of SV_{40} obtained from GMK tissue culture preparations was determined in the fluid harvested from cells

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maintained at 37°C or 40°C in conventional or D_2O (25%) medium. The results (Table II) indicate that the yield of virus was always greater from cells grown in conventional (H_2O) medium. Relative virus yields at 37°C (as $\log_{10}$ PFU/1 ml) were 6.2 ± .06 *vs* 5.0 ± .19 in fluid harvested at 5 days; 5.8 *vs* 5.1 at 9 days and 6.5 *vs* 5.0 at 11 days for H_2O-grown and 25% D_2O-grown cells, respectively.

One explanation of the D_2O effects upon the interaction of SV_{40} with GMK cells (reduced virus yield, lower endpoint titers and reduced plaque size) could be that SV_{40} is inactivated at a greater rate in D_2O-containing medium. To test this possibility a suspension of SV_{40} was incubated for 8 days at 37°C in control and in D_2O (25%) medium. The 2 pools thus obtained yielded the same amount of virus upon titration. These data suggest that the D_2O effect is probably related to virus replication rather than virus inactivation.

The mechanism by which D_2O enhances the replication of poliovirus is not known. Speculations(1-5) on the mode of action have included effects on hydrogen bonding in the nucleic acid and protein as well as effects upon other structural aspects of the virus. The effects of D_2O upon the replication of SV_{40} (inhibition *vs* enhancement of replication at both 37° and 40°C) are markedly different from those observed with poliovirus. A major difference between these 2 viruses is that SV_{40} is a DNA virus while poliomyelitis is an RNA virus. However, studies of D_2O effects upon the *E. coli*-bacteriophage system(10) indicated an enhancement of multiplication when T7 was used and some inhibition of multiplication with T5. More will have to be learned about the effects of D_2O upon various DNA and RNA-containing viruses before these effects may be related to the type of nucleic acid involved. The comparative size of the viruses in question—poliovirus is one of the smallest known RNA viruses, whereas SV_{40} is a large DNA virus—may also be of consequence.

TABLE I. Effect of D_2O (50%) upon Plaque Formation by SV_{40}.

Exp No.	Overlay	Temp	Titer in $\log_{10}$ PFU on day 11	12	13
1	Control	37°C		6.5	6.7
	D_2O	"		1.7	3.7
2	Control	"	6.6		7.5
	D_2O	"	5.1		6.0
3	Control	"	7.4		7.5
	D_2O	"	6.9		7.2
4	Control	"	6.8	7.5	7.6
	D_2O	"	3.7*	6.7	7.2
5	Control	"	6.2	6.8	7.0
	D_2O	"	3.7*	5.8	6.1
6	Control	"	6.4	6.7	6.9
	D_2O	"	<5.7	<5.7	<5.7
7	Control	40°C		6.6	6.7
	D_2O	"		6.4	6.4
8	Control	"	6.4	7.0	7.2
	D_2O	"	6.0	6.3	6.5

* Generalized degeneration and pinpoint plaques observed on monolayers at the 10^{-3} dilution. Monolayers inoculated with SV_{40} diluted beyond 10^{-3} had neither plaques nor degeneration on that particular day. See Fig. 1.

TABLE II. Comparative Virus Yields from Cultures Infected with SV_{40} and Maintained in D_2O (25%) or H_2O Medium.

Exp No.	Day harvested	Temp (°C)	Medium	Titer in $\log_{10}$ PFU per 1 ml of harvest fluid
1	5	37	Control	6.3
		37	D_2O	5.7
2	5	37	Control	6.3
		37	D_2O	<4.7
		40	Control	6.2
		40	D_2O	4.7
3	11	37	Control	6.5
		37	D_2O	5.0
		40	Control	5.8
		40	D_2O	4.7
4	5	37	Control	6.0
		37	D_2O	<4.7
	9	37	Control	5.8
		37	D_2O	5.1
		40	Control	5.7
		40	D_2O	4.7

Summary. The influence of D_2O (25-50%) upon multiplication of and plaque formation by Simian $Virus_{40}$ has been studied in Grivet monkey kidney tissue at 37°C and 40°C. At both temperatures presence of 25-50% of D_2O in the growth medium results in reduced virus yield, lower endpoint titer and reduced plaque size. The effect is not due to inactivation of the virus by deuterium oxide.

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Environmental Effects on Experimental Aerogenic Tuberculosis in BCG-Immunized and Non-Immunized Guinea Pigs.* (28429)

IGNATIUS L. TRAPANI and MAURICE L. COHN
(Introduced by G. Middlebrook)
Division of Research and Laboratories, National Jewish Hospital, Denver, Colo.

Identification of the physiological and biochemical mechanisms through which nonspecific stress may influence immunological responses has not yet been satisfactorily accomplished. The influence of environmental factors on establishment of acute or chronic disease is uncertain because sufficient basic information relative to the host-parasite interaction is lacking.

Studies of antibody formation in rabbits immunized with soluble antigens and exposed to environmental extremes or placed in endocrine imbalance(1,2,3) and work which indicated that animals subjected to simulated altitudes are more resistant to infection by virus but less resistant to bacterial infections (4), led us to investigate further the role of environment in controlled infection. The use of a quantitative system for estimation of tuberculosis infection and disease in guinea pigs induced by the airborne route(5) would satisfy several criteria. The aerogenic route of infection would closely approximate the "natural" one, relatively small numbers of animals could be used for the statistical evaluation of the results, and information might be obtained concerning the amount of disease and environmental influence on the *in vivo* growth of the infecting organism. In contrast to many experiments in which reliance is placed on subjective histological studies and gross pathology for interpretation of the data, the present study utilizes objective methods for quantitation of the extent of the tuberculous process. Previous work by Middlebrook(5) and collaborators (6,7) has indicated these advantages and pointed out that the quantitative method was not only time-saving, but more important, circumvented the effects of allergy and disease itself on the immunity produced by the vaccine(7).

Methods. Animals. Male and female adult guinea pigs weighing 400-600 g were used. These animals were taken from our own random-bred colony of albino guinea pig (Trapani-Campbell strain). The diet of each group was the same. Control and high-altitude groups were kept on dried prairie grass in similar cages; the cold-exposed group was kept on wire screen with no bedding. Each group consisted of 30 guinea pigs, and 3 animals were housed in each cage.

A) The control group was maintained in our Denver Laboratories (altitude = 5,280 ft; temperature = 20°C).

B) The cold-exposed group was housed in a specially constructed cold-box capable of maintaining an environmental temperature

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