An unresolved question is, what causes the rats to stop drinking and perform the reinforced response? Interoceptive stiumulation from the ingestion of water may provide the necessary discriminative cues, but this remains to be determined (*7*).

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References and Notes

1. Distributions of time between responses on spaced-response schedules are given in E. F. Segal, *J. Exptl. Anal. Behav.* **4**, 263 (1961) and M. Sidman, *Science* **122**, 925 (1955).
2. A. Bruner and S. H. Revusky, *J. Exptl. Anal. Behav.* **4**, 349 (1961); M. P. Wilson and F. S. Keller, *J. Comp. Physiol. Psychol.* **46**, 190 (1953).
3. W. Hodos, G. S. Ross, J. V. Brady, *J. Exptl. Anal. Behav.* **5**, 473 (1962).
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5. J. L. Falk, *Science* **133**, 195 (1961).
6. We have not measured daily water intake of individual rats, but we find that 100 ml per day is ordinarily sufficient for a group of three rats caged together and allowed free access to food. Average water intake for rats of 1 ml per hour is reported in the *Handbook of Biological Data*, W. S. Spector, Ed. (Saunders, Philadelphia, 1956), p. 355. Falk (*5*) reports intakes of about 25 ml per day per rat on free-feeding, and intakes of about 100 ml for food-deprived rats during 3-hour experimental sessions of variable-interval dry-food reinforcement.
7. Supported by grant G 18132 from the National Science Foundation (E.F.S.) and by an NSF undergraduate summer research training grant (S.M.H.).

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Tumors Induced in Hamsters by Simian Virus 40: Persistent Subviral Infection

Abstract. *The cells of two hamster ependymomas, originally induced by intracerebral inoculation with simian virus 40 in newborn animals, have been serially cultured in vitro. None of the virus was detected in cell-free culture fluids or cell lysates. All cells retained their neoplastic potential when newborn hamsters were inoculated. Data suggest that viral nucleic acid is permanently present in some tumor cells.*

In extracts of ependymomas induced in newborn hamsters by simian virus 40 (SV_{40}) we were consistently unable to detect the virus, but we could detect it in intact tumor cells seeded onto sensitive indicator cells (*1*), thus the virus may exist in an altered state in some tumor cells. Subsequent work has supported this hypothesis.

Two cell lines, EP_A and EP_H established from two primary ependymomas induced in hamsters with SV_{40} (*1*) were cultivated in vitro for 55 and 35 passages during a period of 14 months. The EP cells were grown in Eagle's basal medium (*2*), 5 percent calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). This medium was free of any detectable inhibitors of SV_{40}. Primary cultures of African green monkey kidneys and rabbit kidneys were grown in a medium consisting of 0.5 percent lactalbumin hydrolyzate, 5 percent calf serum, penicillin, and streptomycin (as above) in Hanks' balanced salt solution. All cell cultures were maintained with Eagle's medium and 2 percent calf serum.

Cultures of EP cells which had not been cloned consisted mainly of epithelial cells, occasional fibroblastic cells, and numerous multinucleated giant cells. The cells exhibited a high mitotic activity and had a generation time of approximately 48 hours. The length of time population cultures can be maintained—2 to 4 weeks with periodic changes of medium—depends on the density of the cell. During the past 14 months, cell-free fluids and lysates of EP cell cultures have been tested repeatedly for SV_{40} with a 28-day assay in monkey kidney cells.

We have been unable to detect infectious virus in such samples, nor did fluorescent antibody tests reveal viral antigen in EP cells. These cells retained their neoplastic potential when newborn hamsters were inoculated.

When cells are placed in contact with sensitive indicator cells from monkey kidney characteristic cytopathogenic effects (CPE) appear within 18 to 20 days in these cells which are adjacent to colonies of EP cells. The virus in these mixed cultures was identified as SV_{40} by neutralization tests with specific antiserum. The initial localization of the CPE near the EP cells and the consistent absence of intra- or extracellular virus in EP cell cultures suggested an inheritable cell-to-cell transfer of infectious viral material, presumably viral deoxyribonucleic acid (DNA). To test this hypothesis the following experiments were carried out:

1) Rabbit kidney cell cultures were infected with SV_{40} at a multiplicity of 0.5 $TCID_{50}$ per cell (tissue culture infective dose—50 percent effective). After a 2-hour adsorption period at 37°C, the inoculum was removed and frozen for assay for residual virus. A sample of the inoculum before adsorption was also frozen. The infected rabbit kidney cell sheet was thoroughly washed, incubated for 14 days, and frozen for assay. Duplicate rabbit kidney cell cultures were infected with phenol-extracted DNA obtained from strain 777 of SV_{40} which originally induced these hamster tumors (*3*). After removal of the inoculum by repeated washing the rabbit kidney cells which had been infected with DNA were incubated for 14 days and frozen for assay. The virus titration in monkey kidney cells was observed for 21 days. Rabbit kidney cells failed to support virus growth after infection with intact SV_{40} (Table 1). By contrast, exposure of these cells to infectious viral DNA resulted in synthesis of small amounts of virus. Viral DNA treated with deoxyribonuclease, 5.0 μg/ml for 10 minutes at room temperature destroyed its infectivity.

Table 1. Results of infection of rabbit kidney cell cultures with intact SV_{40} or infectious viral DNA.

Preparation	$Log_{10}TCID_{50}$/0.2 ml
Intact SV_{40} before adsorption	5.2
Intact SV_{40} 2 hours adsorbed to RK	5.1
RK infected with intact SV_{40}	*
Viral DNA, original inoculum	4.3
RK infected with viral DNA	1.5
RK infected with viral DNA pre-treated with 5.0 μg/ml of DNAase	*

* Noninfective when tested undiluted 0.5 ml/tube.

2) Milk dilution bottle cultures of rabbit and monkey kidney, as well as milk dilution bottles without cells (glass), were seeded with approximately 20,000 EP_{A50} or EP_{H29} cells (50th and 29th passage). In addition, 2×10^7 cells of EP culture were lysed by 10 cycles of freezing and thawing, and the cell-free fluids were tested for virus content (Table 2). No virus was detectable in the lysates of EP cells or of the uninoculated controls. In contrast, significant amounts of SV_{40} were recovered from rabbit, as well as monkey kidney cells.

Additional experiments gave the following results: (i) no activity comparable to that of interferon (*4*) could

Table 2. Results of inoculation of intact or lysed tumor cells. Cell-free lysates of 2×10^7 tumor cells inoculated onto both monkey (GMK) and rabbit (RK) kidney cells were negative. Uninoculated cell controls for both monkey and rabbit kidney were also negative.

Tumor cell line	20,000 intact tumor cells seeded onto*		
	GMK	RK	Glass
EP_{A50}	5.17†	1.97†	Negative
EP_{H29}	5.67	2.17	Negative

* Cells or lysates inoculated into milk dilution bottle cultures. At 10 days, dilutions of the cell-free lysates were subcultured to GMK tube cultures which were held for 21 days. †Total virus $log_{10}TCID_{50}$ present in bottle cultures containing 15 ml medium. Virus was identified as SV_{40} by neutralization tests.

be found in EP culture fluids collected at various intervals; (ii) prolonged passage of EP cells in the presence of high-titer SV_{40} antiserum did not cure the "infection;" (iii) EP cells were resistant to superinfection with SV_{40}. The data strongly suggest that viral nucleic acid is permanently present in some tumor cells. Under certain conditions these cells can introduce this genetic information to indicator cells and thereby initiate viral synthesis. The tumor cells themselves appear to be unable to synthesize infectious virus. There is no evidence to indicate whether the viral nucleic acid is in a proviral or incorporated state (*5*).

The term "virogenic state" may be useful to describe this state. A similar virus-cell relationship may exist in tumors or transformed cells induced by other papova or adenoviruses and perhaps in certain human tumors of suspected viral etiology. Latent viral infections, such as *Herpes simplex*, may persist by a similar mechanism.

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4. Interferon is a nonspecific antiviral protein produced by cells following virus infection.
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15 March 1963

Water Intake of Normal Children

Abstract. *Estimates of daily total fluid intake and of tap-water consumption of normal children were made in four dissimilar geographic areas in the United States. (Total fluid intake increased and, relatively speaking, tap-water consumption decreased, with age.) Of basic importance in fluoridation programs was the observation that even older children rarely drank as much as 500 ml (about 1 pint) of tap water daily.*

Excellent documentation exists in the literature supporting the fact that when drinking water contains 1.0 part of fluoride per million, a marked reduction in the dental-caries rate in children results. Yet the pediatric literature contains surprisingly little reliable information concerning the water intake of normal children. Similarly, systematic data have not been presented in journals devoted to diet and nutrition (*1*). In order to determine the actual daily intake of fluoride necessary for caries reduction, an effort has been made to determine the daily water intake of children.

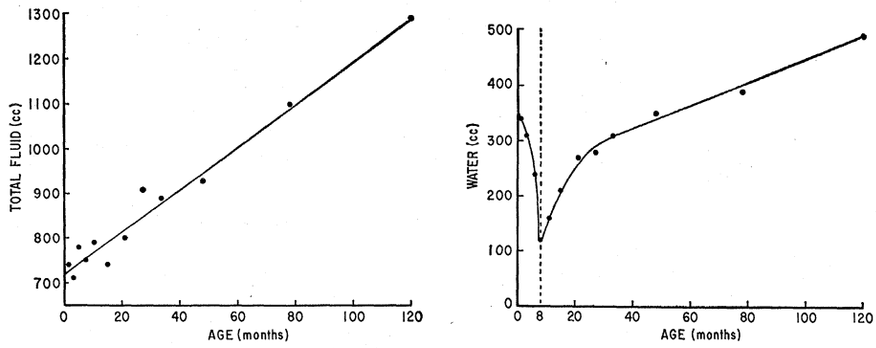

Fig. 1. (Left) Total mean daily fluid intake, by age in months. (Right) Mean tap-water intake per day, by age in months. Note that at all ages, tap water provides less than half of all fluids consumed.

As Neumann has pointed out (*2*), most information on tap water intake in children is in the form of assumptions written "without reference and evident foundation in fact." His own study involved 312 Long Island children under the age of 6. Average daily tap-water consumption, including that added to frozen fruit juices, was 294 ml. The ratio between means for the lowest third and the top third was 1 : 7.4, which emphasizes the variability in tap-water intake among the children studied.

Crosby and Shepherd (*3*), working in Perth with groups of kindergarteners, aged 3 to 5, and school girls, aged 12 to 15 years, also took season of the year into consideration. In both groups fluid intake of water and fluids, mainly water, increased markedly during the summer. The mean volume drunk by the kindergarten group in winter was 319 ml, and this increased to 458 ml per day in summer. Consumption by the older girls averaged 871 ml in winter and 1829 ml in hot weather. Again, volumes of daily water consumption varied considerably among children in the same age groups. In a general way, however, water intake was about the same in the children in the northern United States and those in southern Australia.

Finally, Galagan *et al.* (*4*), in a careful study of climate and fluid intake, found that intake per pound of body weight was highest among infants and decreased with age and that it varied directly with temperature. No substantial differences were observed between boys and girls in the amount of fluid consumed.

In order to learn more about this important feature of childhood behavior, a preliminary study was carried out by one of us (F.J.M.) in Kalamazoo, Michigan, during the 1959–60 school year. Designed to estimate the water intake of normal children, during the fall, winter, and spring of 1960–61, this investigation was expanded to widely separated geographic areas with pronounced climatic differences. It is the purpose of this paper to report our findings and to interpret them.

Altogether, 797 children were studied. Of these, 83 were residents of Miami, Florida; 177, of Atlanta, Georgia; 250, of Los Alamos, New Mexico; and 287, of Kalamazoo, Michigan.

Studies in all four cities were carried out with the protocol developed in 1959–60, as follows. Mothers of infants and children seen in private office

Table 1. Total fluid and water consumed in various age groups (total 797 children).*

Age	Fluid per day (ml)		Water per day (ml)	
	Mean	SE of mean	Mean	SE of mean
Breast-fed children				
0–6 mo	50	17	19	7
Bottle-fed children				
0–2 mo	740	77	342	26
2–4 mo	707	43	314	54
4–6 mo	783	29	238	75
6–9 mo	754	58	120	26
9–12 mo	793	43	160	59
12–18 mo	737	41	208	34
18–24 mo	799	30	267	56
24–30 mo	907	95	283	24
30–36 mo	894	47	312	22
Older children				
3–5 yr	926	33	349	27
5–8 yr	1102	40	389	38
8–12 yr	1290	116	493	191
12 and older	1247	107	301	146

* Data on the four separate cities studied may be obtained from the authors of this report.