replication. It is clear that the data do not fit this line.

It is not clear from Fig. 2 alone, however, that a stable fraction exists whose proportions are those predicted by the semiconservative model. To determine the stable fraction more precisely we had to choose between measuring either a few survival curves for longer storage times, or many survival curves for several cultures, but over shorter storage times. We chose the latter procedure and such data from five sets of experiments comprising a total of 19 inactivation curves, where n ranged from 0.69 to 3.5, are summarized in Fig. 3. The fraction of stable cells, F, obtained from each survival curve, is plotted as a function of the number of cell divisions occurring after labeling (n). There is a general increase in F with increasing n. This is demonstrated by the solid line through the data and means that, for more than one cell division after labeling, a stable fraction of cells exists. This indicates that the mode of replication of DNA is not dispersive regardless of the manner in which the DNA is organized in the cells. A comparison between the predictions of the semiconservative model of Table 1 (which assumes one duplex structure as the minimum DNA content per bacterium) and experiment is also indicated in Fig. 3. The dashed curve A represents the predicted stable fraction according to the relation $F=(2^n-2)/2^n$ whereas the solid line represents the best fit to the experimental data. The deviation of the stable fraction in Fig. 3 from curve A could be due to the fraction of cells that did not contain the minimum amount of DNA when the labeling period ended, but were part way through the synthesis of the second duplex. Analysis in terms of assumptions for numbers of DNA duplexes greater than one is possible. Our results suggest only that for 15_{T-L-}, under our experimental conditions, the simplest model for the organization of DNA is one duplex for cells that have just completed division. The single hit inactivation curves might be taken as further confirmation of this view.

We emphasize that the assay used in these experiments is one of biological function. Thus, it is interesting to compare our results with those previously reported for the segregation of DNA, when this was examined on a structural basis. Our conclusion, that DNA replication in *E. coli* is semiconservative, is in agreement with the density gradient studies of Meselson and Stahl (*8*) on *E. coli*, and with the H^3 autoradiographic studies of Forro and Wertheimer (*9*) on the same organism.

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Oncogenic Effect of Human Adenovirus Type 12, in Mice

Abstract. *Undifferentiated malignant tumors were induced at the site of injection of human adenovirus type 12 into newborn mice of the C_3H_f/G_s strain, but not of the DBA_f or A_f strains. The tumors were grossly and histologically similar to those induced by this virus in hamsters, but appeared in a smaller percentage of injected mice than hamsters.*

Our previous observations of the oncogenic effect of human adenovirus type 12, injected into newborn hamsters (*1, 2*) were confirmed and extended by Huebner *et al.* (*3*). Newborn hamsters were selected for testing the oncogenicity of human viruses because of their greater sensitivity to the oncogenic effect of murine polyoma virus (*4*) than even the mouse, the species of origin of the polyoma virus, and because of their relatively poor defense against the growth of normal tissue and tumor tissue transplants from other species, including man (*5*). Having induced the formation of tumors in hamsters by injection of a particular human virus, we wished to determine whether tumors could be induced by this virus in other species also. We therefore injected adenovirus type 12 into newborn mice of three different strains.

Human adenovirus type 12, strain Huie, was propagated in HeLa cells in our laboratory, and cell-free filtrate from the infected tissue culture was prepared as described previously (*1*). Mice of the C_3H_f/G_s, DBA_f, and A_f

Table 1. Relative tumor incidence in mice and hamsters injected at birth with equal doses of adenovirus type 12.

Route of injection	No. of animals with tumor/ No. of animals surviving more than 30 days	Site of tumor	Age at death from tumor (days)	No. dead without tumors (or missing) *	No. alive without tumors
		Mice of strain DBA_f			
Intrapulm.	0/4			3 (533–544) †	1 (776) †
Intraperit.	0/4			4 (528)	
Subcut.	0/5			4 (536–678)	1 (770)
		Mice of strain A_f			
Intrapulm.	‡0/7			‡3 (183–618)	4 (771)
Intraperit.	‡0/3			‡3 (540–647)	
Subcut.	0/6			4 (474–704)	2 (769–771)
		Mice of strain C_3H_f/G_s			
Intrapulm.	3/7	Thorax	61, 81, 145	3 (261–537)	1 (776)
Intraperit.	1/4	Abdomen	111	3 (466–675)	
Subcut.	0/2			2 (611–663)	
		Syrian hamsters			
Intrapulm.	26/27	Thorax and liver	35 – 157	1 (156)	

* A few mice could not be accounted for. † Ages, in days, given in parentheses. ‡ Two old mice of strain A_f were found at autopsy to have round lung adenomas of the type that occur spontaneously in this strain, but these are not included in the results.

strains, when less than 24 hours old, were injected with 0.05 ml of infected tissue culture fluid. The mice were injected by the intrapulmonary (through the chest wall), intraperitoneal, or subcutaneous route, by means of a 30-gauge needle.

The titer of the fluid used for inoculation was 10^3 $MTCID_{100}$ per 0.1 ml (number of minimum 100 percent infective tissue culture doses), as determined by the specific cytopathic effect on HeLa cell tubes, with final reading on the 5th day (*1*). Van Hoosier, in this laboratory (*6*), found that when other batches of adenovirus type 12 were cultured on primary embryonic kidney tissue from humans, the titers on the 20th day were 1×10^4 times higher than those on the 5th day when the virus was cultured on HeLa cells.

Whereas tumors developed at the site of injection of the virus in 26 of 27 hamsters, as reported earlier (*1*), in the mice no tumors were found in strains DBA_2 or A_2, but 4 out of 13 mice of the strain C_3H_f/G_s developed tumors at the site of injection (Table 1). Histologically, these tumors were undifferentiated, and were similar in both gross and microscopic appearance to those induced in hamsters (*1*). In a subsequent group of C_3H_f/G_s mice injected intraperitoneally with adenovirus type 12 at birth, 12 of 17 died of induced tumors in from 56 to 155 days. The histology of the tumors, their development at the site of injection at a relatively early age, and the absence of such tumors among hundreds of mice of this strain in our colony leave little doubt that the tumors were induced by injection of adenovirus type 12. Also, like the tumors in hamsters, the tumors in mice were transplantable. Huebner *et al.* (*3*) have recently reported tumor induction in three of ten newborn rats injected with adenovirus type 12. The oncogenicity of this human virus is therefore demonstrable in at least three species.

Tumors induced by polyoma virus (*7*), Simian virus-40 (*8*), and the Gross leukemia virus (*9*) contain "cancer-specific" antigenicity. The tumors induced in hamsters by adenovirus type 12 apparently also contain "cancer-specific" antigenicity. This is indicated by the requirement of approximately ten times more tumor cells to achieve the same percentage of successful tumor transplants in hamsters preimmunized with adenovirus type 12, as in normal hamsters (*10*). However, for such quantitative transplantation studies, the tumors induced in mice by the same virus, when transplanted to a histoisogenic inbred strain, represent a much more ideal system than the hamster tumors, arising as they do in non-inbred animals.

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Circularity of the Replicating Form of a Single-Stranded DNA Virus

Abstract. *Electron micrographs of purified replicating form DNA of the bacteriophage ϕX-174 show that it has ring structure. The circular property may explain both the abnormally high sedimentation coefficient and the great resistance of this DNA to irreversible heat denaturation. Intactness of the circle is required for the control of strand selection during transcription into complementary copies of RNA.*

It has been shown by Sinsheimer *et al.* (*1*) that subsequent to injection the single-stranded DNA of the bacteriophage ϕX174 is converted into a replicating form (RF). Hayashi *et al.* (*2*) succeeded in purifying the RF-DNA by repeated chromatography on columns of methylated albumin coated on kieselguhr (MAK). The resulting preparations were free of detectable contamination by either the host DNA or the single-stranded DNA of the mature virus particle. The buoyant density of RF-DNA in cesium chloride and its melting temperature agree (*2*) with that expected from a double-stranded DNA structure containing 43 percent guanine-cytosine. We now show that intact RF-DNA has a ring structure, which explains certain unique properties that differentiate this DNA from the fragmented rod preparations commonly examined.

A peculiarity of the RF-DNA is its great resistance (*1*, *2*) to denaturation by the usual heating and fast-cooling procedures. Another paradoxical feature was revealed in the current study in the course of measuring its sedimentation constant. Figure 1*A* exhibits the sedimentation pattern of RF-DNA purified by chromatography and Fig. 1*B* shows the effect of high-frequency sound on the same preparation. The sharp boundary of the RF-DNA indicates a high degree of homogeneity. On the other hand, the sound-treated preparation shows the usual blurred boundary characteristic of

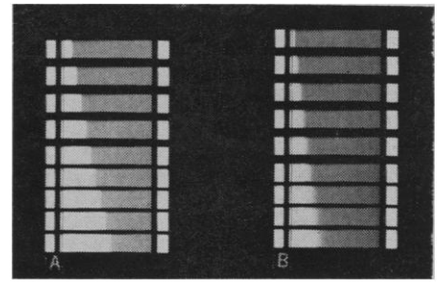

Fig. 1. *A*, Sedimentation pattern of RF-DNA [35 μg/ml in 0.15*M* NaCl, 0.015*M* sodium citrate (SSC), at 22°C]. Pictures were taken every 4 min at 44,770 rev/min. *B*, Sedimentation pattern of RF-DNA (20 μg/ml in SSC at 22° C.) treated with high frequency sound with a Raytheon sonic oscillator for 6 min at 4°C. Pictures taken every 8 min at 44,770 rev/min.