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

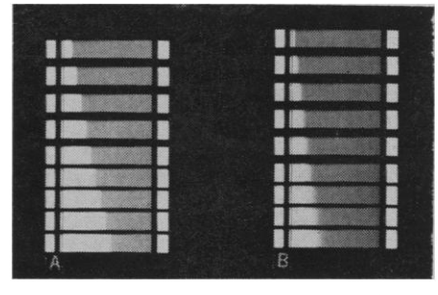

Fig. 1. *A*, Sedimentation pattern of RF-DNA [35 μg/ml in 0.15*M* NaCl, 0.015*M* sodium citrate (SSC), at 22°C]. Pictures were taken every 4 min at 44,770 rev/min. *B*, Sedimentation pattern of RF-DNA (20 μg/ml in SSC at 22° C.) treated with high frequency sound with a Raytheon sonic oscillator for 6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

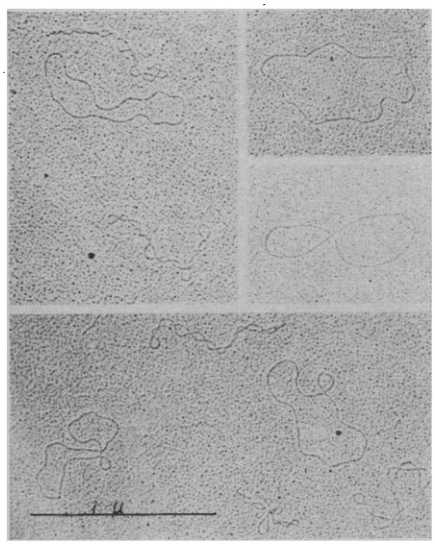

Fig. 2. Electron micrographs of ϕX174 RF-DNA. The method of Kleinschmidt was used for electron-microscope preparations (*15*). A protein salt solution (2*M* ammonium acetate. 0.01% cytochrome *c*, *p*H 7) containing 2μg of DNA per millimeter was spread on a water surface. After the film had ceased to expand, grids with formar carbon membranes were touched to the monolayer, then touched to absolute ethyl alcohol for 20 seconds, and drained on filter paper. The preparations were shadowed with 12 mg of uranium oxide at 11 cm and an angle of 6°. Grids were rotated during shadowing. Pictures were taken with a Siemens Elmiskop IIb at 50 kv. The magnification was calibrated with a carbon replica of a diffraction grating (*16*).

fragmented DNA preparations. Calculation of the $S_{20,w}$ of the untreated RF-DNA yielded a value of 21; the sound-treated preparation had an average $S_{20,w}$ of 8. A sedimentation constant of 21 corresponds (*3*) to a molecular weight of 6.4×10^6 which is approximately twice that expected from Sinsheimer's (*4*) estimation of the size of the single strand (1.7×10^6). This discrepancy suggests that RF-DNA diverges from the usual rod-shape of DNA. This possibility is made more likely by the enzymatic data of Fiers and Sinsheimer (*5*) which suggested a circular structure for the single-stranded DNA from which the RF-DNA is derived.

Since pure preparations were available, they were examined by electron microscopy. Figure 2 shows clearly that intact RF-DNA is a circular structure. Figures 3*A* and *B* are electron micrographs of the DNA employed in sedimentation experiments of Fig. 1, Fig 3*A* corresponding to the untreated RF-DNA and Fig. 3*B* to the sound-treated sample. It is clear that treatment with high-frequency sound ruptures the circular structure and produces rods.

The abnormally high sedimentation coefficient of RF-DNA may, therefore, be explained by the greater compactness of the circular structure compared to that of a rod of equivalent length. The denaturation properties may ultimately also be explained by some as yet unspecified peculiarity of the ring. For example, a circular double-stranded helical structure which did not contain an even number of reversals in the helix would not separate on denaturation into two free circular single strands. The strands would remain intertwined and hence renaturation would readily occur. Dulbecco and Vogt (*6*) as well as Weil and Vinograd (*7*) have recently found very similar properties for the DNA derived from the mature particles of the polyoma virus.

The length of the circular structures (Fig. 2) was 1.89 ± 0.04 μ. On the assumption that one is dealing with a Watson-Crick double helix, this length corresponds to approximately 5600 base pairs and is in good agreement with the estimation of 5500 bases in the single strand of DNA found (*4*) in the mature virus particle.

The concept of a circular DNA is not new. It first emerged as a brilliant deduction in Jacob and Wollman's (*8*) interpretation of their data on recombination in *Escherichia coli*. Subsequently, similar genetic evidence for circularity was provided (*9*) by experiments with the bacteriophage T4. More direct evidence for the ring structure was provided by radioautographs of *E. coli* (*10*) and electron micrographs of DNA from bacteriophage λ (*11*) as well as from polyoma virus (*7*).

The RF-DNA of ϕX174 is one of the more interesting DNA preparations available for experimental study. Because of its small size extraordinary precautions are not required to avoid fragmentation during extraction and purification. By chromatographic procedures (*2*) it is relatively simple to prepare pure RF-DNA which, on examination by a variety of devices, is composed of over 95 percent intact rings.

Hayashi, Hayashi, and Spiegelman (*12*) sought to inquire whether the intact ring structure could explain the mystery of why RNA messages are generated from only one (*13*) strand of the DNA in the cell and from both strands in vitro (*14*). Samples of RNA synthesized with intact and ruptured rings as templates were compared by hybridization (*12*) and analysis of nearest neighbor to guanine. The results were clear-cut. Intact circles generated RNA strands complementary to only one of the two strands of the RF-DNA, the strand which is complementary to the one formed in the mature virus particles. This is the same strand shown (*13*) to be used in the intact cell to generate genetic messages. Fragmented circles produced RNA strands, complementary to both. It would appear that strand selection (for message production) is a built-in feature of the intact circular DNA molecule.

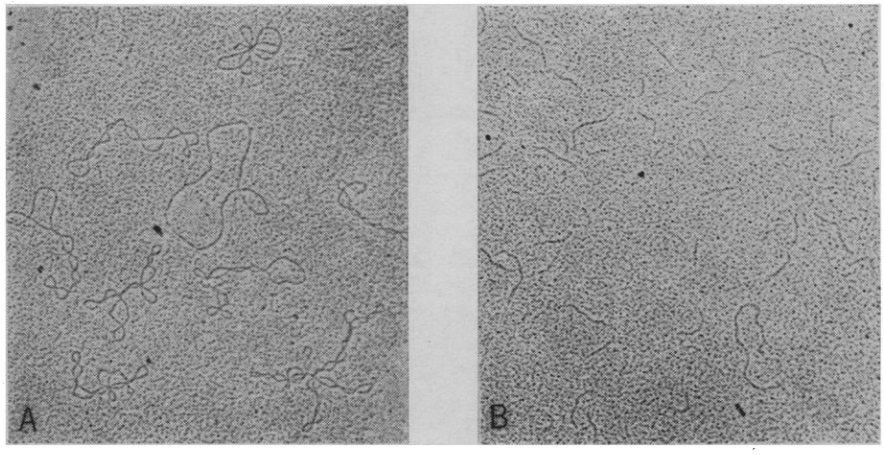

Fig. 3. Electron micrographs of ϕX174 RF-DNA before (*A*) and after (*B*) treatment with high-frequency sound. The samples are identical with those shown in Figs. 1*A* and *B*, respectively, and the pictures were taken by the method described for Fig. 2.

An equally informative analysis of the replication of DNA in vitro is also provided by the existence of this material.

Note added in proof: After this manuscript was submitted for publication, two papers appeared, providing evidence for the circularity of RF-DNA of ϕX147 (*17*).

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Erythropoietin Production Following Gamma Irradiation and Hemorrhage in Dogs

Abstract. *The production of erythropoietin in dogs increased after they had been exposed to 300 rad of gamma rays and then subjected to hemorrhage.*

The recovery of erythropoiesis which takes place in irradiated rats and dogs, and the possible role of erythropoietin as a stimulating factor in these irradiated animals has been discussed (*1*). In particular, it has been noted that bleeding has a favorable effect in irradiated animals (*2*). We thought it possible that one of the factors contributing to this favorable effect might be the increased titers of erythropoietin following bleeding. We therefore conducted experiments to test this hypothesis.

Nine mongrel dogs, each weighing approximately 10 kg, were divided into three groups. Group I was irradiated and immediately thereafter bled until 30 percent of the total blood volume was removed; group II was irradiated but not bled; group III was bled without prior exposure to radiation. All irradiated dogs received 300 rad of a 2 kc Co^{60} source over the whole body. During irradiation the animals were placed in specially designed cages providing a constant geometry and sufficient oxygen supply. The dogs were bled by cardiac puncture; blood for assay was taken from veins in the hind leg. Serum was separated by centrifuging at about 1200 *g* for 30 minutes.

For comparison, one female dog which had not been irradiated was made anemic by injections with a 2.5 percent aqueous solution of phenylhydrazine hydrochloride, a total of 35 ml being given over a 7-day period. The initial hemoglobin level of 20.2 g/100 ml of blood decreased to 9.8 g/100 ml. Serum was prepared as for the other dogs. Erythropoietic activity of plasma was assayed by a modified version of the method described by Plzak *et al.* (*3*).

Female Wistar albino rats (numbering 180) were divided into groups of five. Each rat was ready for use when it weighed approximately 160 g; all were starved for 24 hours before use, and until they were killed. Three intraperitoneal injections of 2 ml of dog serum were given in the course of 3 days. Six hours after the third injection, 0.5 μc of protein-bound Fe^{59} ferric citrate was injected; 19 hours later the uptake of Fe^{59} by the blood was measured in a scintillation counter with a 5-percent mean counting error. The erythropoietic activity was expressed as the percentage of Fe^{59} taken up, assuming the total blood volume of rats to be 5 percent of the body weight.

The response of the three groups of dogs differed in both time and magnitude of the apparent level of erythropoietin (Table 1).

The maximum response shown by the dogs in group III was attained on the second day; the level of erythropoietin had returned to normal by the 6th day. Group II showed no response until the 8th day, the uptake of Fe^{59} in the injected, fasted rats increasing to a maximum on the 12th day and declining slowly thereafter. In contrast, the dogs in group I showed a greater response than either group II or III, and the response was biphasic (Fig. 1). In the first phase, which began on the second day, the maximum uptake occurred on the 4th day; in the second phase, the maximum occurred on the 18th day. In magnitude, the second phase in the response of group I was comparable to that of the dog injected with phenylhydrazine; the serum of this dog, when assayed in ten rats, resulted in an Fe^{59} uptake of 13.27 $\pm$ 8.28 percent.

Table 1. The uptake of Fe^{59} in rats injected with sera from dogs that were irradiated and bled (group I); irradiated but not bled (group II); bled but not irradiated (group III). Each number represents the mean value from five rats $\pm$ standard deviation. Results obtained with untreated control rats were 3.71 $\pm$ 1.55; 3.78 $\pm$ 2.14; 3.00 $\pm$ 0.09; 2.68 $\pm$ 1.03; 3.36 $\pm$ 0.63 .

Day	Group I	Group II	Group III
0	3.03 $\pm$ 0.47	2.83 $\pm$ 0.03	2.68 $\pm$ 0.57
2	6.60 $\pm$ 2.46	3.61 $\pm$ 0.85	6.57 $\pm$ 1.23
4	12.43 $\pm$ 3.41	3.71 $\pm$ 0.72	4.94 $\pm$ 1.31
6	5.08 $\pm$ 1.71	2.98 $\pm$ 0.50	3.09 $\pm$ 0.93
8	5.17 $\pm$ 1.16	4.78 $\pm$ 1.36	2.71 $\pm$ 0.56*
12	5.98 $\pm$ 1.45	7.40 $\pm$ 2.75	3.06 $\pm$ 0.69*
13	7.85 $\pm$ 2.43	6.60 $\pm$ 0.49	2.67 $\pm$ 0.16
16	8.48 $\pm$ 2.91	5.44 $\pm$ 2.25	2.17 $\pm$ 0.35*
18	10.22 $\pm$ 2.19	4.88 $\pm$ 2.44	2.44 $\pm$ 0.25
21	9.87 $\pm$ 4.53		3.38 $\pm$ 1.05*

* One day before or after the indicated period.

Assuming that the assay method used here provides a reliable measure of the circulating level of erythropoietin, it is apparent that the irradiated dog is capable of producing erythropoietin in response to hemorrhage. It is significant that the dogs which were both irradiated and bled (group I) showed

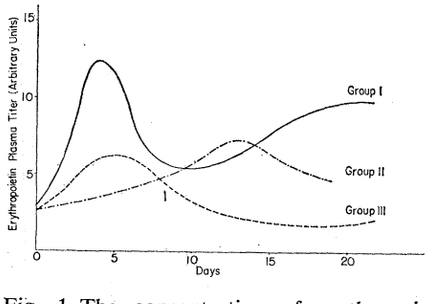

Fig. 1. The concentration of erythropoietin in the serum of dogs irradiated with gamma rays and then bled (group I); irradiated but not bled (group II); bled but not irradiated (group III).