and its substitution by tris-citrate buffer (*p*H 8.5) immediately after the color developed.

When stained, controls lacking substrate (FDP or F-1-P) developed no color. The spot produced by purified aldolase from rabbit muscle corresponded with that located by the amido-black protein stain.

The aldolase isozymes seen thus far are shown in Figs. 1, 2, and 3. We have numbered the spots from anode to cathode. Five isozymes were detected in man, seven in the rat, and at least four in the frog. The aldolase of frog skeletal muscle shows an extensive unresolved band which may consist of more than one component. The rat is peculiar in that its tissues apparently contain seven isozymes, two of which, when subjected to electrophoresis on starch gel, migrate toward the cathode. The species variation can clearly be seen in Fig. 3. While, for a given species, all isozymes may be present in each tissue, their proportions vary, each organ having a typical, reproducible pattern.

It is conceivable that other faint bands of activity were not detected because the total aldolase activity of the different homogenates varied. However, the homogenates of each tissue (at several concentrations, as determined by protein content) have been examined by electrophoresis, and we have found no more than five isozymes in any animal tissue except rat tissue. If the method can be made more sensitive, other forms may possibly be found.

One of the methods used to distinguish between the two previously described forms of aldolase is based on their different affinites for substrates. The "liver aldolase" described by Blostein and Rutter (*2*) has a much higher affinity for F-1-P than does the "muscle aldolase." When F-1-P is substituted for FDP as substrate, only isozymes L-5 and K-5 (Fig. 3) can be detected in human tissue homogenates. In rat tissue homogenates, similar F-1-P substitution shows L-6 and 7 and K-6 and 7 only. Hence, we feel that these isozymes represent the "liver aldolase" described previously. It appears that the patterns of these isozymes in liver and kidney are closely similar.

Previous evidence suggests that aldolase comprises three polypeptide subunits (*3, 6*). More recently, data from detailed studies with the ultracentrifuge have strongly suggested the occurrence of four subunits (*8*), and results of biochemical investigations lend additional support to this view (*9*). The occurrence of five isozymes of aldolase could be more easily explained if there were four polypeptide subunits rather than three; by analogy, one may cite the example of lactate dehydrogenase in this context. The two negatively migrating isozymes detected in the rat are difficult to explain. They are, however, consistently found, and they exhibit activity with either FDP or F-1-P as their substrate.

HAROLD B. ANSTALL
CAROL LAPP
JOSE M. TRUJILLO
Department of Pathology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston

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Allomorphic Forms of Bacteriophage φX-174 Replicative DNA

Abstract. *The bacteriophage φX-174 replicative form of DNA exists in two configurations, as judged by electron microscopy and sucrose density gradient sedimentation, and on methylated albumin–coated kieselguhr column chromatography. The predominant 21*S *form of the DNA is a tightly twisted circle. Treatment with deoxyribonuclease causes a single scission in one of the two strands, resulting in an open circle which sediments at 16*S.

Two classes of DNA molecules are observed when the bacteriophage φX-174 replicative double-stranded form of DNA (RF-DNA) is isolated from its bacterial host (*1*). When this RF-DNA was purified by sucrose density-gradient sedimentation, it separated into two components. Approximately 90 to 95 percent of these molecules sedimented with an s_{20} value of 21; the remainder, with 16. These two forms could also be separated by a column of methylated albumin–coated kieselguhr (Fig. 1). Both appeared to be almost equally infective on *Escherichia coli* spheroplasts, and they had an identical buoyant density in neutral CsCl. These data agree with those reported by Jansz and Pouwels (*2*).

Similar results were obtained with SV40 viral DNA (*3*) and with polyoma DNA (*4*). With the polyoma virus, Vinograd *et al.* (*4*) demonstrated that the faster-sedimenting 20*S* polyoma DNA molecules have a highly twisted circular form. They and others (*2*) demonstrated that, upon exposure to very dilute pancreatic deoxyribonuclease, one of the two strands was cleaved and the molecules sedimented at 16*S*. Examination of the 16*S* fraction in the electron microscope showed that the molecules were open circles. On the basis of the polyoma data, we thought it likely that the two differently sedimenting forms of φX-174 RF-DNA also might be due to a similar shift in conformation. As shown below, this is the case.

When purified 21*S* RF-DNA was treated with very dilute pancreatic deoxyribonuclease, the concentration of the 21*S* form decreased with a con-

Table 1. Deoxyribonuclease conversion of 21*S* to 16*S* DNA. Purified 21*S* RF-DNA was treated with pancreatic deoxyribonuclease (reaction mixture as in Fig. 2). At the intervals noted, samples were analyzed by electron microscopy (Fig. 3) and sucrose-gradient sedimentation (Fig. 2).

Treatment time (min)	No. of molecules counted in electron micrographs			21*S* by sedimentation (%)
	Open form	Closed form	Percentage closed	
0	307	3370	91.7	95
2	343	2190	86.5	78
5	1312	999	43.2	50
10	3310	710	17.6	26
15	2855	287	9.1	13
20	3053	149	4.6	6

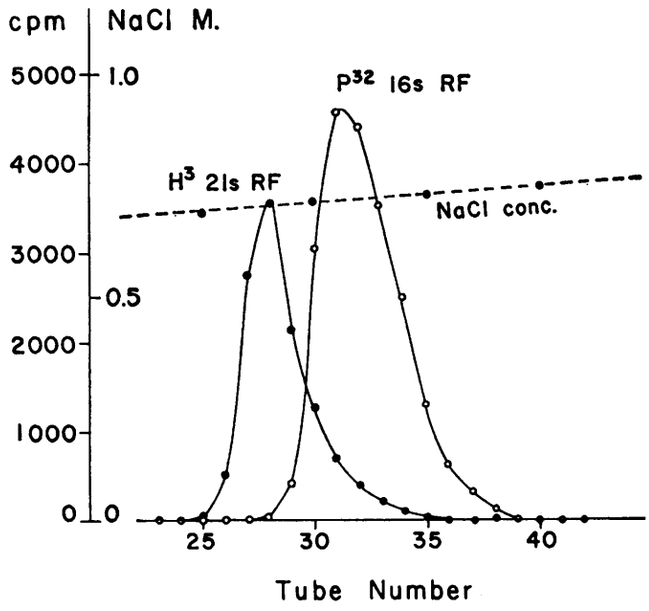
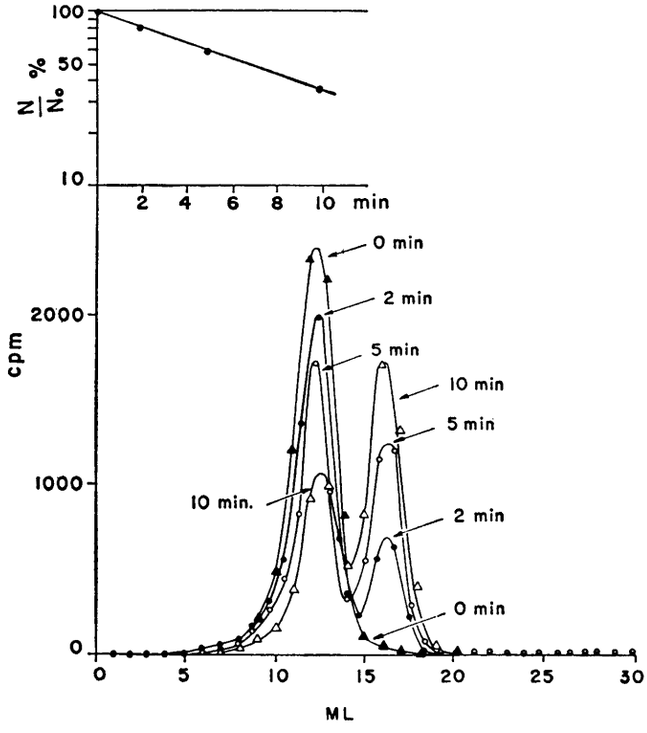


Fig. 1. Elution of tritiated 21*S* RF-DNA and P^{32}-labeled 16*S* RF-DNA from a methylated albumin–kieselguhr column by a NaCl gradient. The DNA was labeled and isolated separately from ϕX-174–infected cells and purified by sucrose-gradient sedimentation before being mixed and placed on the column.



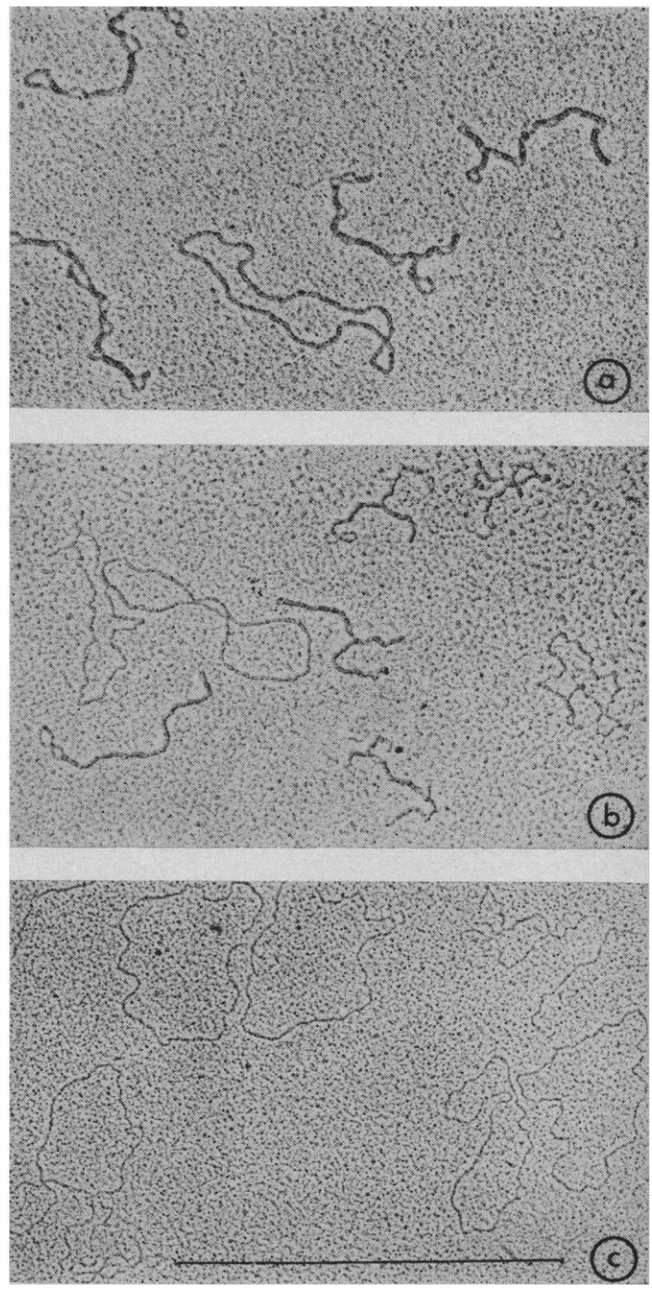


Fig. 2 (bottom left). Conversion of 21*S* to 16*S* RF-DNA after exposure to pancreatic deoxyribonuclease. The reaction mixture contained (per milliliter) : 20.5 μg of H^3-labeled 21*S* RF-DNA, 0.0004 μg of pancreatic deoxyribonuclease, 10 μmole of $MgCl_2$, 30 μmole of tris-HCl, *p*H 7.2. The reaction was performed at 25°C. At the times indicated, samples (0.2 ml) were taken and mixed with 0.2 ml of 0.2*M* ethylenediamenetetraacetate (*p*H 8.0), 0.15*M* NaCl, and 0.1 percent duponol (final concentration). Samples were layered on sucrose gradients (20 to 5 percent by weight in $3 \times 10^{-2}M$ tris-HCl, *p*H 7.3, and 0.1*M* NaCl) and centrifuged at 25,000 rev/min for 14 hours at 5°C (SW 25.1 rotor; Spinco L-2 centrifuge). In the upper scale the fraction of 21*S* RF-DNA that has survived deoxyribonuclease action (N/N_o) is plotted against time of the enzyme treatment. Fig. 3 (right). Electron micrographs of ϕX RF-DNA in various stages of conversion from 21*S* to 16*S* molecules after progressively longer periods of exposure to deoxyribonuclease. Micrographs a, b, and c are representative fields taken of samples after 0, 4, and 20 minutes with deoxyribonuclease (see Table 1). The DNA (about 10 μg/ml) in parts a and b was spread in trypsin treated with diisopropyl fluorophosphate and heavily shadow-cast with uranium; in part c, the DNA was spread in cytochrome-c and lightly shadow-cast. The scale line represents 1.0 μ.

comitant increase in the 16*S* form as shown by sucrose density-gradient analysis (Fig. 2). Since this shift follows first-order kinetics, we assume that a single scission in one of the two strands produces the conversion.

In a similar experiment, samples were taken at several intervals during the incubation with deoxyribonuclease and assayed for the relative amount of 21*S* and 16*S* classes both by sucrose-gradient centrifugation and by counting from electron micrographs according to Kleinschmidt and Zahn (*5*) and Weil *et al.* (*6*). The predominantly 21*S* form present at the beginning of the experiment (Fig. 3a) is converted to a mixture of open and closed forms in 5 minutes (Fig. 3b). After 20 minutes, most molecules are in the 16*S* open form (Fig. 3c). When scoring, only tightly twisted forms were counted as closed; others, merely loosely overlapping a few times, were considered already cleaved by treatment with deoxyribonuclease, and thus we regarded them as open (Table 1).

The lower percentage of closed circles scored by direct count as compared to the numbers calculated from sedimentation data (Table 1) probably is due to a conversion of the 21*S* to the 16*S* form by unknown factors during the 1- to 2-day interval between treatment with deoxyribonuclease and the preparation for electron microscopy. Both the open and loosely twisted molecules had an average length of 1.77 ± 0.08 μ.

Thus, we conclude that (i) the 21*S* molecules are tightly twisted circles, (ii) the 16*S* class are open circles, and (iii) a single scission in one of the two strands of DNA converts the 21*S* to the 16*S* form. However, at this time we are unable to state whether or not the 16*S* form occurs naturally in the host bacterium because of the ease with which the 21*S* molecule is converted to the 16*S* form (*7*).

THOMAS F. ROTH
MASAKI HAYASHI
Department of Biology, University of California, San Diego, La Jolla

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7. The purified 16*S* fraction from *E. coli* infected with ϕK-174 was examined with an electron microscope. Ninety-six percent of the 16*S* form consisted of open or loosely twisted circles similar in appearance to those that had been exposed to deoxyribonuclease (Fig. 3c), and no more than 4 percent were noncircular.
8. Supported in part by USPHS grants GM 12934-02 and AI-04255. We thank Edna Fuller for help in scoring the electron micrographs.

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Reversal Response Elicited in Nonbeating Cilia of Paramecium by Membrane Depolarization

Abstract. *Ciliary reversal occurs in response to electrical and chemical stimuli in specimens of* Paramecium caudatum *in which ciliary beat has been completely inhibited by external application of nickel ions. The mechanism underlying ciliary reversal appears, therefore, to differ from that of ciliary beat. The cessation of ciliary beat has no effect on the intracellular potential of* Paramecium. *However, depolarizing action potentials are associated with ciliary reversals in paramecia, treated with nickel, without ciliary beat. Thus, membrane depolarization in this species seems specifically concerned with the ciliary reversal, and not with ciliary beat.*

Ciliary beat of *Paramecium caudatum*, a ciliated protozoan, is completely inhibited by external application of nickel ions (*1*). The nonbeating cilia thus obtained can reverse their orientation in response to chemical and electrical stimuli (Fig. 1). This is thought to be the same response as the reversal phenomenon of normally beating cilia (*2*, *3*). Ciliary reversal seems to be a phenomenon analogous to the coupling of excitation and contraction in muscles.

When the potassium concentration in the external medium is adequately increased, *Paramecium* temporarily (90 seconds or more) swims backward because the direction of the effective ciliary beat is transiently reversed (*3*, *4*). The response is called "ciliary reversal" or "reversal response of cilia." Ciliary reversal occurs in response to stimuli other than chemical, such as electrical and mechanical. Galvanic current through the cell membrane elicits a consistent ciliary reversal on the cathodal surface of the organism. When a forward-swimming specimen strikes a solid object, it swims backward a distance, then starts to swim forward in a new direction. This behavior is known as the "avoiding reaction" and can also be seen in an interface be-

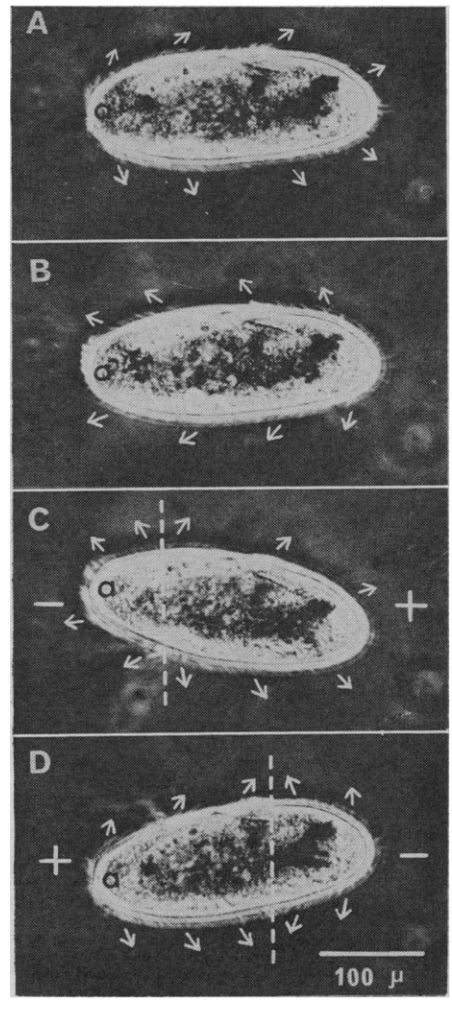

Fig. 1. Responses of cilia to potassium ions and electric current in a *Paramecium caudatum* whose ciliary beat is completely inhibited by nickel ions applied externally. (A) Without stimulus; nonbeating cilia point in normal direction. (B) Responses of nonbeating cilia to an increase in external potassium ions; orientation of cilia is reversed. (C and D) Responses of nonbeating cilia to electric current (applied voltage is 4.5 volt/cm); the specimen was held with its longitudinal axis slightly oblique to the lines of stimulating current. Reversal of orientation of cilia occurs at the cathodal side of the cell (cilia on the left side of white dotted line on the specimen in C and on the right side in D). Small white arrows indicate the approximate pointing direction of cilia near the arrows. Anterior side of the organism is marked *a*.