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*).

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References and Notes

1. B. Chandler, M. Hayashi, M. N. Hayashi, S. Spiegelman, *Science* **143**, 47 (1964); A. K. Kleinschmidt, A. Burton, R. L. Sinsheimer, *ibid.* **142**, 961 (1963).
2. H. S. Jansz and P. H. Pouwels, *Biochem. Biophys. Res. Commun.* **18**, 589 (1965).
3. L. V. Crawford and P. H. Black, *Virology* **24**, 388 (1964).
4. J. Vinograd, J. Lebowitz, R. Radloff, R. Watson, P. Laipis, *Proc. Natl. Acad. Sci. U.S.* **53**, 1104 (1965).
5. A. K. Kleinschmidt and R. K. Zahn, *Z. Naturforsch.* **14b**, 770 (1959).
6. R. Weil, J. Vinograd, W. Stoeckenius, *Proc. Natl. Acad. Sci. U.S.* **50**, 730 (1963).
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.

8 September 1966

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-

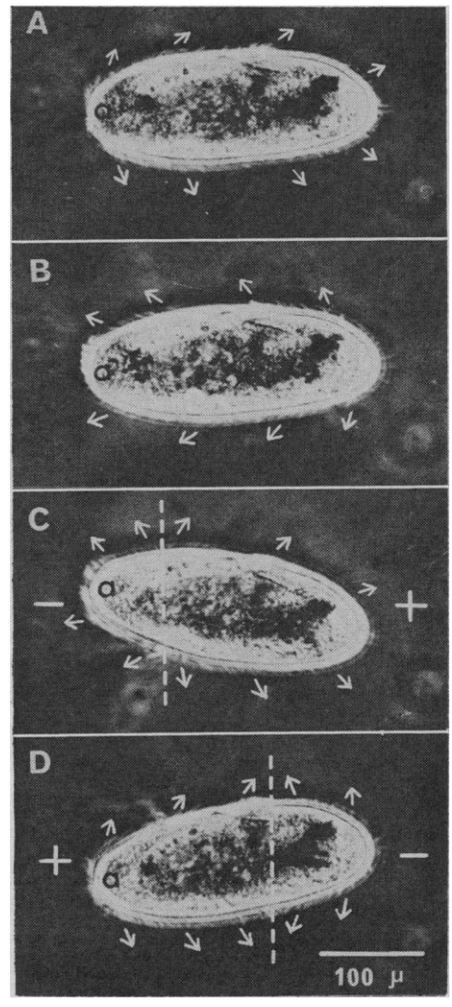

Fig. 1. Responses of cilia to potassium ions and electric current in a *Paramecium caudatum* whose ciliary beat is completely inhibited by nickel ions applied externally. (A) Without stimulus; nonbeating cilia point in normal direction. (B) Responses of nonbeating cilia to an increase in external potassium 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*.

tween two different solutions (*3*). A number of investigations of ciliary reversal related to cell excitation were summarized by Jahn *(4, 5)*. However, the fundamental mechanism underlying the response remains unclear.

Paramecium caudatum (*6*) (200 to 250 μ in length) reared in hay infusion was washed well with a solution containing 2 mmole of KCl, 1 mmole of $CaCl_2$, and 5 mmole of tris(hydroxymethyl)aminomethane (tris) buffer (*p*H 7.2) per liter and was equilibrated for 30 minutes or more in the solution before experimentation.

For microscopical observations and photographic recording of ciliary position, a phase-contrast objective was focused on an organism immobilized at the tip of an inserted microneedle (less than 0.5 μ in tip diameter) in a trough on the stage of a microscope. All the experiments were performed at room temperatures of 20° to 21°C.

Treatment of *Paramecium* with a nickel solution [5 m*M* $NiCl_2$, 2m*M* KCl, 1 m*M* $CaCl_2$, and 5 m*M* tris buffer (*p*H 7.2) in final concentration] brought about a gradual decrease in both the frequency and the amplitude of the ciliary beat and finally caused its cessation within about 3 minutes. *Paramecium* treated with nickel exhibited no ciliary beat for about 30 minutes or more, even after complete removal of nickel ions from the external solution. All experiments on nonbeating cilia were performed in the nickel-free equilibration medium before ciliary beat resumed.

Figure 1A is a photomicrograph of a *Paramecium* treated with nickel. Almost all the nonbeating cilia point posteriorily, which is the direction of effective beat during forward swimming in the normal organism.

When a *Paramecium* treated with nickel was exposed to a solution rich in potassium [20 m*M* KCl, 1 m*M* $CaCl_2$, 5 m*M* tris buffer (*p*H 7.2) in final concentration] the orientation of its nonbeating cilia was consistently reversed (Fig. 1B) in a forward-pointing direction, corresponding to the reversed beat direction of the backward-swimming organism. Subsequently, the cilia gradually returned to their original orientation. The duration of the reversal response of the nonbeating cilia was 36 ± 1.0 seconds [mean and standard error (S.E.) of five measurements on five different specimens]—almost the same as the duration of backward swimming, in normal *Paramecium*, induced by the same increase in external potassium concentration (37 ± 1.5 seconds; five measurements on five specimens).

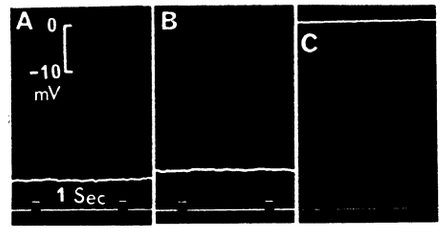

Fig. 2. Intracellular potentials of *Paramecium caudatum*. (A) Before treatment with nickel; all cilia beat normally and small irregular fluctuations in potential level were seen. (B) After treatment with nickel; the ciliary beat had completely ceased and potential fluctuations similar to those in A were noted. The slightly smaller potential level is probably not significant, for in some specimens the potential increased and in others remained unchanged by treatment with nickel (see text). (C) after the death of the specimen there is neither a transmembrane potential difference nor any potential fluctuations. Calibration and zero-level of the potential are shown in A. An interval between two square pulses in the lower traces corresponds to 1 second.

Electric current applied through a pair of parallel platinum electrodes (5 mm by 5 mm) to a *Paramecium* treated with nickel brought about a distinct reversal in the pointing direction of nonbeating cilia on the cell surface near the cathode (Fig. 1, C and D). The boundary between two areas on the cell surface, bearing reversely pointed cilia (cathodal side) and normally pointed cilia (anodal side), seemed to fall in a plane at about 90° to the flux line of the stimulating current. The nature of the response to electric current is identical, except for the lack of ciliary beat, to those in normal paramecia, described by many authors since Ludloff *(2, 5)*. Current intensities necessary to induce a just-perceptible reversal response of cilia at the anterior and directed toward the cathode were determined for both normal and nickel-treated paramecia and were found to be the same (3.2 ± 0.12 volt/cm in normal organism; 3.2 ± 0.07 volt/cm in nickel-treated organism: means and S.E. of five measurements on five different specimens).

These results demonstrate that in *Paramecium* the reversal of cilia in resonse to cathodal current and potassium ions occurs independently of ciliary beat and that nickel ions act specifically to inhibit ciliary beat.

In saponified or glycerinized models of *Paramecium*, ciliary beat occurred in the presence of adenosine triphosphate (ATP) and magnesium ions at *p*H 6.5 *(7)*, whereas ciliary reversal without ciliary beat occurred when ATP and

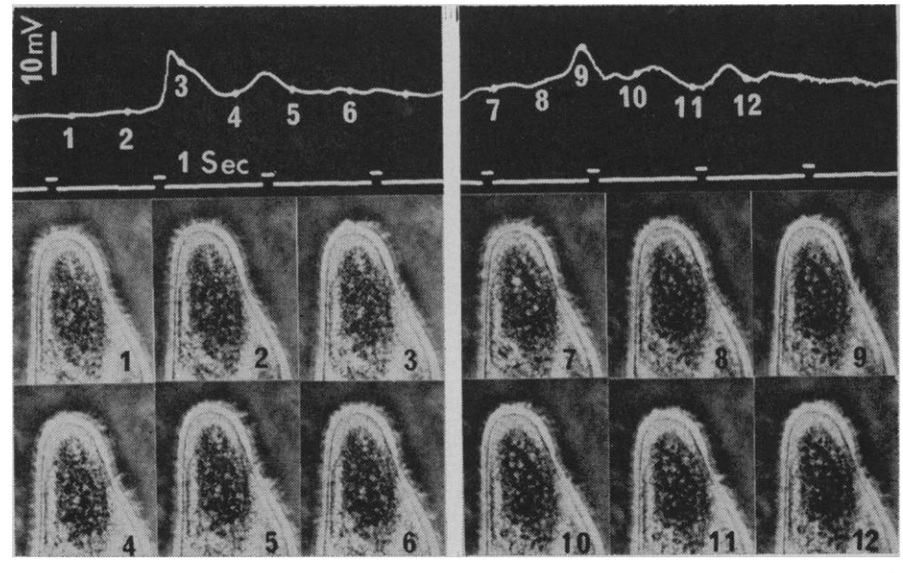

Fig. 3. Two samples of simultaneous recordings of spontaneous depolarization and ciliary reversal in nickel-treated *Paramecium caudatum* without ciliary beat. A nickel-treated specimen was kept in a solution with a low concentration of calcium (0.01 m*M*) so that spontaneous electrical activity of the membrane might be induced. Numbered white dots on the upper potential traces show each moment when correspondingly numbered photographs of the specimen were taken (magnification, approximately × 140). Strong ciliary reversals associated with large depolarizations are observable. (See 3 and 9. Notice the cilia on the right side of the specimen and compare them with those in 1 and 7.) Weak ciliary reversals were associated with depolarizations of small magnitude (see 4, 5, and 10). An interval between two square pulses on the lower traces shows 1 second.

calcium ions were applied at *p*H 8.7 (*8*). These facts strongly support Worley's hypothesis that contractile systems concerned with beat and with reversal may be separable and distinct from each other (*9*).

In another series of experiments, a glass microcapillary electrode (about 0.5 μ in tip diameter) filled with 0.1*M* NH_4Cl (*10*) was introduced into cytoplasm of *Paramecium*, and the potential difference between the inserted electrode and a reference electrode placed in the external solution was amplified, displayed on a cathode-ray tube, and photographed.

Penetration of the microelectrode into cytoplasm of a normal *Paramecium* was generally accompanied by a sudden shift to a negative direct current level in recorded potential ranging from −15 to −40 mvolt (*10, 13*). Sometimes small, irregular, repetitive fluctuations in potential level (less than 1 mvolt) were found (Fig 2A). Intracellular potentials of a similar nature were also recorded from a nickle-treated *Paramecium* with nonbeating cilia (Fig. 2B). Therefore, the small perturbations in potential level would appear to have no relation to ciliary beat. Moreover, the degree of the inside negativity in nickel-treated *Paramecium* (21 ± 1.0 mvolt; mean and S.E. of five measurements on five different specimens) was found to be almost equal to that in normal *Paramecium* (20 ± 0.27 mvolt; mean and S.E. of five measurements on five different specimens). In contrast, in the ciliated cells of the ctenophore comb plate, depolarizing action potentials are associated not only with natural ciliary beats but also with beats initiated in response to mechanical vibrations of cilia (*11*).

Low concentrations of extracellular calcium (*12*) frequently cause spontaneous reversal in the ciliary beat of normal specimens (*12*). Similar spontaneous reversals of orientation occur in nickel-treated specimens in media with a low calcium concentration as does nickel-treated *Paramecium* without ciliary beat. Spontaneous reversals in normal specimens are accompanied by transient membrane deplorizations (*10, 13*). Recordings of intracellular potentials of nickel-treated *Paramecium* made simultaneously with photographic recording of spontaneous reversal responses of nonbeating cilia in 0.01 m*M* $CaCl_2$ (Fig. 3) show that the reversal response of nonbeating cilia are always associated with depolarizations of the membrane.

It is concluded that membrane depolarization is specifically concerned with the ciliary reversal and not with ciliary beat in *Paramecium*.

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References and Notes

1. L. Kuznicki, *Acta Protozool.* **1**, 301 (1963); T. L. Jahn and E. C. Bovee, in *Research in Protozoology*, T. T. Chen, Ed. (Pergamon Press, London, 1966), p. 40.
2. K. Ludloff, *Pflüger's Arch. Ges. Physiol.* **59**, 525 (1895).
3. H. S. Jennings, *Behavior of the Lower Organisms* (Columbia Univ. Press, New York, 1906), p. 62.
4. T. L. Jahn, *J. Cell. Comp. Physiol.* **60**, 217 (1962).
5. ———, *J. Protozool.* **8**, 369 (1961).
6. I thank Dr. K. Hiwatashi of Tohoka Univ. (Sendai) who identified the specimen as Mating Type I of Syngen 1.
7. L. N. Seravin, *Biokhimiya* **26**, 160 (1961).
8. Y. Naitoh, in preparation.
9. L. G. Worley, *J. Cell. Comp. Physiol.* **5**, 53 (1934).
10. T. Yamaguchi, *J. Fac. Sci., Univ. Tokyo, Sec. IV*, **8**, 573 (1960).
11. G. A. Horridge, *Nature* **205**, 602 (1965).
12. A. Grebecki, *Acta Protozool.* **3**, 275 (1965).
13. H. Kinosita, A. Murakami, M. Yasuda, *J. Fac. Sci., Univ. Tokyo, Sec. IV*, **10**, 421 (1965.)
14. I thank Dr. R. Eckert of Syracuse Univ. for critical reading of the manuscript.

26 July 1966

Arrested Protein Synthesis in Polysomes of Cultured Chick Embryo Cells

Abstract. *Cells deprived of serum synthesize proteins at a reduced rate; when serum is restored the rate returns to normal. The polysomes do not dissociate, but show reduced incorporation of amino acid in vitro, and are less responsive to polyuridylic acid than are those from normal cells.*

In primary cultures of chick embryo cells, protein synthesis shows an absolute requirement for serum (*1*): in its absence there is no detectable net synthesis of protein or RNA, and incorporation of precursors into these classes of macromolecules is sharply curtailed. The three principal classes of RNA—ribosomal, messenger, and soluble—continue to be synthesized but at a very reduced rate (*2*).

In order to identify the phase of protein synthesis that requires serum, the effect of serum withdrawal from cells that are synthesizing protein at the maximum rate was explored. The results suggest (i) that polysomes remain intact when deprived of serum; (ii) that messenger RNA (mRNA) is not destroyed; and (iii) that these polysomes are relatively ineffective in incorporating amino acids in vitro.

Cells cultured as monolayers in Eagle basal medium (*3*) with 3 percent calf serum synthesize protein for several days, the rate of growth being linear (*1*). When the medium was replaced by one without serum at any time during the linear phase of growth, the net protein and RNA syntheses ceased abruptly (Fig. 1). The amount of cellular protein and RNA decreased during the next 24 hours by approximately 20 percent, probably representing cells which had become detached from the surface.

When serum was added to the cells at any time for several days after its initial removal, the rate of protein synthesis increased rapidly (Figs. 1A, and 2), and within 4 hours the rate was equal to that of cells maintained in serum. An unusually high initial rate of protein synthesis is often observed under such circumstances (*4*). For 24 hours after serum had been restored, the net amount of protein synthesized was essentially normal.

When actinomycin D was added with the serum, after the cells had been deprived of serum for various periods, the initial rate of protein synthesis (that during the first 4 hours) (Fig. 2), was not adversely affected. It thus appears that when serum is absent mRNA is present but not translated.

These results could be due to persistence or to renewal of mRNA while the cells were deprived of serum. To distinguish between these possibilities, actinomycin was used to prevent renewal of mRNA during incubation without serum. The capacity for protein synthesis was then tested after the serum was restored. This capacity slowly decreases during 16 hours (Fig. 3), but the decrease was even faster in identically treated cultures incubated with both actinomycin and serum. It appears that depriving cells of serum impairs the translation of mRNA but does not impair, and even promotes, the persistence of RNA in functional form. Although the effect of actinomy-