development of the fertilized sea urchin egg. The antiserum to 5-acetyluracil diluted 1 : 10 allowed the embryos to hatch (blastular stage) before further development was arrested. These blastulas continued movement for 48 hours. The effects of a 1 : 100 dilution of this antiserum were identical to those seen with a 1 : 1000 dilution of the antipurinoyl serum. A 1 : 10 dilution of antiserum to adenylic acid led to the formation of abnormal blastulas which were not viable, whereas a 1 : 100 dilution of the same antiserum allowed pluteus formation. The effects of antiserums to uridylic acid and to nicotinamide adenine dinucleotide at 1 : 10 dilutions were similar to those seen with a 1 : 10 dilution of the antiserum to adenylic acid.

The antiserums prepared against the conjugate of polyuridylic acid and bovine serum albumin slowed the developmental cycle by a time factor of approximately 2. Eventually the embryos reached the late gastrular prismatic stage, at which time further development stopped in spite of continued viability for 3 days. The relative effectiveness of these antiserums at comparable dilutions was not a function of their content of precipitating antibody as measured by addition of the homologous antigens after absorption of antibody to protein. Whether the observations are related to specificity of interaction with the DNA of *Arbacia* remains to be clarified.

Tyler has reported (*7*) that the development of fertilized sea urchin eggs is blocked by antibodies to surface constituents, but not by antibodies directed against internal antigens. The experiments reported here demonstrate that antiserums to purines and pyrimidines can affect embryonic development. In view of the specificity of these serums, that is, their reactions with thermally denatured DNA, and the observations with fluorescein-labeled antibodies, the most reasonable explanation would be that antiserums can penetrate the embryo and become fixed within the cell. The results also provide evidence for the existence in vivo of DNA molecules that are either totally or partially single-stranded.

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References and Notes

1. See, for example, F. Lipmann, in *Progress in Nucleic Acid Research*, J. N. Davidson and W. E. Cohn, Eds. (Academic Press, New York, 1963), vol. 1, p. 135.
2. A. Kornberg, *Science* **131**, 1503 (1960).
3. V. P. Butler, Jr., S. M. Beiser, B. F. Erlanger, S. W. Tanenbaum, S. Cohen, A. Bendich, *Proc. Natl. Acad. Sci. U.S.* **48**, 1597 (1962); S. W. Tanenbaum and S. M. Beiser, *ibid.* **49**, 662 (1963); B. F. Erlanger and S. M. Beiser, *ibid.*, in press.
4. The antiserums were diluted in sea water and kept in contact with the eggs for 1 hour. The cells were then collected by gentle centrifugation, washed three times with sea water, and examined directly in the fluorescence microscope. A portion of the fertilized embryos was allowed to develop further (until stopped by the specific inhibitory effect of the anti-serum) to demonstrate the viability of the cells after contact with the antiserum.
5. Prepared by Dr. K. C. Hsu of this Department by a previously described method: A. H. Coons and M. H. Kaplan, *J. Exptl. Med.* **91**, 1 (1950) as modified by J. L. Riggs, R. J. Seiwald, J. H. Burckhalter, C. M. Downs, T. G. Metcalf, *Am. J. Pathol.* **34**, 1081 (1958). We thank Dr. Hsu for making these specimens available to us.
6. I. B. Weinstein and C. Wilde, in preparation.
7. A. Tyler, *Am. Zool.* **3**, 109 (1963).
8. R. Chambers and M. J. Kopak, *J. Cellular Comp. Physiol.* **9**, 331 (1937); *ibid.*, p. 345; N. L. Gershfeld, *Biol. Bull.* **125**, 362 (1963).
9. Eggs in sea water were fertilized with a dilute suspension of fresh sperm collected according to the procedure of E. B. Harvey, *The American Arbacia and Other Sea Urchins* (Princeton University Press, Princeton, 1956). The embryos were allowed to develop at 20°C. The antiserums were added only after the formation of the fertilization membrane. Serums obtained from the rabbits before immunization served as controls.
10. Aided by grants from the NSF and USPHS, and by contracts between the Office of Naval Research and Columbia University. The authors benefited greatly from discussion of this work with Dr. A. Bendich.
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Electrophorus Adenosine Triphosphatase: Sodium-Activated Exchange after N-Ethyl Maleimide Treatment

Abstract. *The microsomal fraction from the electric organ of the eel* Electrophorus electricus *catalyzes the hydrolysis of adenosine triphosphate in the presence of Mg^{++}, Na^{+}, and K^{+}. The same preparation catalyzes a Mg^{++}-dependent transphosphorylation between adenosine triphosphate and adenosine diphosphate. Both of these reactions are inhibited after treatment of the microsomes with* N*-ethyl maleimide. However, the addition of Na^{+} reactivates the transphosphorylation, and the rate becomes more rapid than that of the original. This new Na^{+}-sensitive exchange reaction is believed to be a component of the hydrolytic reaction.*

Several studies have implicated a specific adenosine triphosphatase in the cell membrane as a component of "active Na^{+} transport" (*1*). This hydrolytic enzyme was initially found in the microsomal fraction of crab nerve (*2*) and has since been found in many tissues. It is most active in the tissues of the nervous system and in tissues concerned with secretory function (*3*). This enzyme reaction shares many features with the process of active sodium transport, including a specific requirement for adenosine triphosphate (ATP), localization at the cell membrane, inhibition by cardiac glycosides, and a concurrent requirement for Mg^{++}, Na^{+}, and a second monovalent inorganic cation. The specificity for sodium appears to be absolute for the activation of the adenosine triphosphatase (to be referred to here as Na^{+}-K^{+}-ATPase).

The microsomes of crab nerve also catalyze a transphosphorylation of the terminal phosphate from ATP to ADP (exchange reaction) (*4*). From this association of activities Skou postulates a high-energy phosphorylated intermediate of the Na^{+}-K^{+}-ATPase. The exchange reaction requires Mg^{++}, and it is slightly inhibited by Na^{+}. The microsomal fraction from the electric organ of *Electrophorus electricus* contains a similar triphosphatase (*5*) and also catalyzes an ATP-ADP exchange reaction which requires only Mg^{++} (*6*); Na^{+} and K^{+} have no effect on the exchange reaction.

Skou has studied the effects of sulfhydryl inhibitors on this phosphatase from ox brain (*7*). Both *p*-chloromercuribenzoate and *N*-ethyl maleimide inhibited the enzyme activity. We have recently studied the effects of the maleimide on *Electrophorus* microsomes in detail. Although the triphosphatase and the Mg^{++}-dependent exchange activities are both inhibited by it, the exchange rate can be reactivated by the addition of a low concentration of Na^{+}.

The enzyme was prepared as described (*6*). It was incubated with $10^{-3}M$ *N*-ethyl maleimide in $0.1M$ tris - hydroxymethylaminomethane - HCl buffer (tris) at *p*H 8.0 at 0°C for 30 minutes, at which time excess maleimide was removed by transferring a portion into seven volumes of 0.005

Table 1. Response of ATP-ADP exchange activity to NaCl after treatment with *N*-ethyl maleimide (NEM). After the preincubation described in the text, the samples were again incubated for 30 minutes at 26°C with 0.003*M* $MgCl_2$; 0.005*M* tris ATP; 0.0012*M* tris ADP-C^{14}; 0.04*M* tris-HCl, *p*H 7.5; 1.5 μg of microsomal protein in experiment 1 and 2.5 μg in experiment 2; additional salt was added as indicated; the total volume was 25 μl. Control refers to enzyme incubated with H_2O in place of *N*-ethyl maleimide. Under these conditions of the maleimide treatment, adenosine triphosphatase activity is inhibited by about 80 percent.

Additions	Exchange rate (% of control)		
	Expt. 1	Expt. 2	Untreated with NEM
None	23	17	100
$5 \times 10^{-5}M$ NaCl	26		
$1 \times 10^{-4}M$ NaCl	28		
$5 \times 10^{-4}M$ NaCl	60		
$1 \times 10^{-3}M$ NaCl	126		
$2 \times 10^{-3}M$ NaCl	250	312	110
$1 \times 10^{-2}M$ NaCl		800	88
$5 \times 10^{-2}M$ NaCl		500	98
$1 \times 10^{-1}M$ NaCl		450	
$5 \times 10^{-2}M$ LiCl		20	
$5 \times 10^{-2}M$ KCl		18	

percent β-mercaptoethanol in 0.05*M* tris at *p*H 7.5. From this solution, portions were transferred to tubes containing substrates for the exchange reaction. The separation of the radioactive nucleotides was accomplished by a thin-layer chromatographic method.

The calculation of exchange rate (Table 1) is based on the percent of total radioactivity in ATP; a series of control enzyme dilutions was run with each experiment to obtain a standard curve, upon which the exchange rate was plotted. In the control experiments, equilibration occurred to an extent of 18 to 22 percent of the total radioactivity in ATP, corresponding to an exchange rate of approximately 228 mμmole of phosphorus per minute per milligram of protein (*8*).

Treating the particles with *N*-ethyl maleimide inhibits the Mg^{++}-dependent exchange activity about 80 percent (Table 1). The original rate of exchange, however, can be restored by less than $10^{-3}M$ NaCl; in contrast, the reaction catalyzed by untreated particles is insensitive to Na^+. With increasing concentrations of NaCl, the rate of the exchange reaction becomes several fold greater than that of the original Na^+-insensitive exchange. This suggests that Na^+ is not merely reactivating the original Mg^{++}-dependent exchange. Since the marked response to Na^+ is specific (Li^+ and K^+ are ineffective, Table 1), it is reasonable to consider that this Na^+-sensitive exchange may be a component of the *Electrophorus* Na^+-K^+-ATPase. The ATP-ADP transphosphorylation implies the occurrence of a high-energy phosphorylated intermediate in the adenosine triphosphatase reaction (presumably enzyme-bound), and the present experiments indicate that the role of sodium ions in both reactions may involve a transfer of high-energy phosphate. Since the Na^+-sensitive exchange is not evident until after the enzyme reacts with *N*-ethyl maleimide, some change in enzyme configuration may be induced by this reagent.

Longer treatment of the microsomes with the maleimide inactivates the adenosine triphosphatase more completely, but it also begins to inhibit the Na^+-activated exchange rate. There is no significant incorporation of radioactivity from ADP into AMP with control enzyme or maleimide-treated enzyme. The enzyme preparation had previously been found to be virtually free of adenylate kinase activity (*6*).

It should be emphasized that the evidence for the identification of this exchange reaction as a component of the hydrolytic reaction remains purely circumstantial.

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References

1. R. L. Post, C. R. Merritt, C. R. Kinsolving, C. D. Albright, *J. Biol. Chem.* **235**, 1796 (1960); E. T. Dunham and I. M. Glynn, *J. Physiol.* **156**, 274 (1961); R. Whittam, *Biochem. J.* **84**, 110 (1962); S. L. Bonting and L. L. Caravaggio, *Arch. Biochem. Biophys.* **101**, 37 (1963).
2. J. C. Skou, *Biochim. Biophys. Acta* **23**, 394 (1957).
3. S. L. Bonting, K. A. Simon, N. M. Hawkins, *Arch. Biochem. Biophys.* **101**, 37 (1963).
4. J. C. Skou, *Biochim. Biophys. Acta* **42**, 6 (1960).
5. R. W. Albers and G. J. Koval, *Life Sciences* **1**, 219 (1962).
6. R. W. Albers, S. Fahn, G. J. Koval, *Proc. Natl. Acad. Sci. U. S.* **50**, 474 (1963).
7. J. C. Skou, *Biochem. Biophys. Res. Commun.* **10**, 219 (1963).
8. H. A. C. McKay, *J. Am. Chem. Soc.* **65**, 702 (1943).

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Root Pressure in Conifers

Abstract. *Exudation of sap was never observed to occur from stumps of detopped seedlings of loblolly pine or white spruce, but measurable exudation occurred from apical root segments, 4 to 8 centimeters long, which were removed from the root systems and observed individually. Fully suberized root segments of loblolly pine exuded as much or more sap than unsuberized roots. Exudation also occurred from detached sugar maple root segments, but not from stumps attached to entire root systems.*

Exudation rarely is observed from stumps of coniferous seedlings or large trees, and it generally is assumed that conifers do not develop root pressure. However, there are enough reports of exudation from conifer roots to indicate that root pressure sometimes is developed. Eaton (*1*) raised *Thuja orientalis* and *Cedrus deodara* in solutions containing high concentrations of salt and, by replacing the salt solution with tap water at the time the tops were removed, he was able to produce exudation. Daniel (*2*) obtained barely measurable volumes of exudate from a single seedling of *Pinus ponderosa* and from two seedlings of *Pinus radiata*. Pitra (*3*) observed exudation from stumps of plants of *Thuja occidentalis, Cupressus horizontalis, Cupressus funebris*, and *Pinus insignis*, but he detected no root pressure exudation in *Juniperus ericoides, Taxus baccata*, or *Picea alba*. Under field conditions exudate has been collected from detached roots of pine (*4*, *5*), spruce (*5*, *6*), and larch (*6*).

Our observations on seedlings of loblolly pine (*Pinus taeda L.*) and white spruce [*Picea glauca* (Moench) Voss] provide new information. The seedlings were grown for several months in a greenhouse in aerated, nutrient solution (*7*) before the experiments were started. Observations were made in the spring, with a root temperature of approximately 24°C. No exudation ever occurred from stumps of detopped seedlings. Likewise, no exudation was ever observed from stumps of detopped seedlings which had been grown in sand or in soil.