Purine- and Pyrimidine-Specific Antibodies: Effect on the Fertilized Sea Urchin Egg

Abstract. *Antiserums from rabbits immunized with several purine- and pyrimidine-containing albumin conjugates can penetrate and become fixed within cells of* Arbacia punctulata. *All of these specific antiserums were found to inhibit embryonic development of the fertilized sea urchin egg. The stage of arrestment of development was dependent in part upon the dilution of antiserum, and also upon the nature of its specificity. It is suggested that the observed results reflect the interaction of these antiserums with single-stranded DNA within the cell.*

Developments in chemical biology suggest that translation of genetic information into the synthesis of specific protein molecules occurs by transmission of the "code" from single-stranded DNA to messenger RNA, which in turn carries the information to the ribosomes (*1*).

An additional role has been assigned to single-stranded DNA by Kornberg and his associates (*2*) who found that such molecules could act as templates in DNA duplication. Thus, evidence has accumulated for the existence of single-stranded DNA within the cell and for its importance in the regulation of biological processes. The direct isolation and demonstration of such DNA molecules is extremely difficult, probably because most biological systems do not consist of synchronously growing cells and, at any one time, only a small portion of the DNA would be single-stranded.

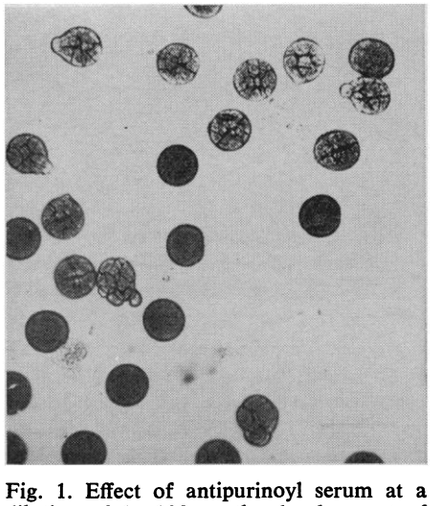

Fig. 1. Effect of antipurinoyl serum at a dilution of 1 : 100 on the development of the fertilized sea urchin embryo, 100 minutes after fertilization.

Immunization of rabbits with proteins conjugated with purine or pyrimidine derivatives induces production of antiserums which cross-react with thermally denatured DNA (*3*). Except for antiserums to the haptene 5-acetyluracil, none reacted with native DNA. We report here the effects of these antiserums on embryonic development of the sea urchin in order to provide evidence for the existence of single-stranded DNA, or possibly of DNA with partially disrupted base pairs, in the cell.

Experiments were carried out to test whether these antiserums could penetrate cells of *Arbacia punctulata*. Unfertilized eggs and eggs 10 minutes after fertilization were exposed (*4*) to fluorescein-labeled antipurinoyl globulin (*5*) with resulting internal localization of the fluorescence. In contrast, control experiments showed that neither nonspecific fluorescein-labeled rabbit globulin (Difco) nor fluorescein-labeled duck antirabbit globulin (*5*) became irreversibly fixed. These results apparently demonstrate that antibodies penetrate living cells. It is not surprising that sea urchin eggs admit molecules the size of gamma-globulin since polyuridylic acid has been reported to exert an effect on such cells (*6, 7*). The observation that sea urchins can assimilate oil droplets half their own size (*8*) may also have a bearing on this point.

In studies of the effects of specific antiserums on the development of the sea urchin embryo (*9*) all of the antiserums tested were found to affect embryonic development. The results with antiserums to purin-6-oyl bovine serum albumin are illustrated in Figs. 1 through 4. Figure 1 shows the abnormal cleavage caused by the action of the antipurinoyl serum, diluted 1 : 100, when added 5 minutes after fertilization. This picture was taken 100 minutes after the addition of the antiserum. Embryos that were not visibly affected by the antiserum at this time developed to the blastula stage, at which point they were arrested prior to hatching (Fig. 2, 24 hours after fertilization). With the same antiserum diluted 1 : 500, the embryos proceeded to the early gastrula stage; further development was stopped. The embryos remained viable at this stage for an additional 48 hours (Fig. 3, 49 hours after fertilization). Differentiation proceeded to the late gastrula stage when exposed to the antipurinoyl serum diluted 1 : 1000. Although development was arrested, the embryos remained viable for at least an additional 48 hours (Fig. 4, 49 hours after fertilization). The possibility that these effects were due to the presence of a toxic principle was eliminated by showing that an antiserum absorbed with purinoyl human serum albumin was inactive. Inhibition identical to that discussed earlier in this report was obtained with the purified fluorescein-labeled antipurinoyl globulin; on the other hand, the nonspecific tagged globulin was inactive.

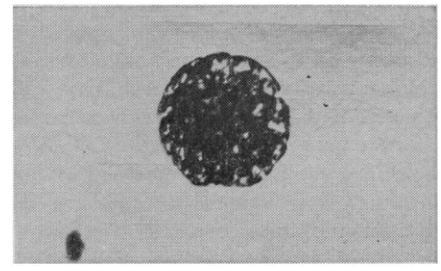

Fig. 2. Effect of antipurinoyl serum diluted 1 : 100 on the developing sea urchin embryo, 24 hours after fertilization.

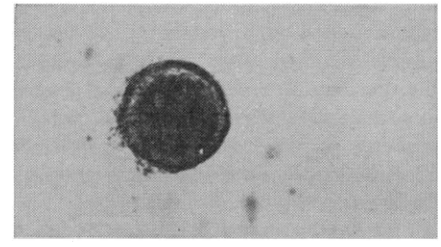

Fig. 3. Effect of antipurinoyl serum diluted 1 : 500 on the developing embryo, 49 hours after fertilization.

Other antiserums to purines and pyrimidines also inhibited embryonic

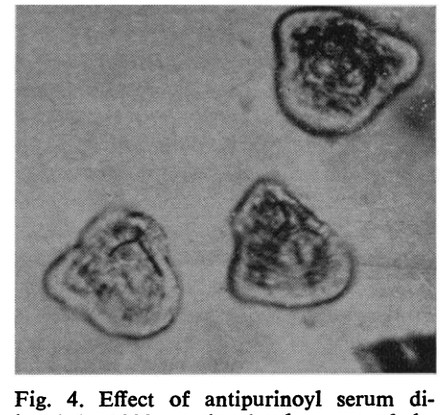

Fig. 4. Effect of antipurinoyl serum diluted 1 : 1000 on the development of the fertilized sea urchin embryo, 49 hours after fertilization.

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