EFFECTS OF ATP AND EDTA ON CLEAVAGE OF *LIMNAEA* EMBRYOS

THAT ATP should play a role in the energetics of cleavage is quite expected in view of its well-known property, namely, providing the energy supply for biological endergonic pro-

cesses. Nevertheless, the results of experiments on the effects of ATP on developing eggs are contradictory. Brachet[1] and Wolpert[2] gave a short summary of such attempts. Wolpert[3] reported that sea-urchin eggs placed in ATP (4×10^{-2} M) ten minutes before cleavage would develop in a delayed, abnormal way or would be arrested altogether but later he[2] could not confirm this result and suggested that it was in artefact. In spite of this, the train of reasoning started by the above authors remains interesting especially in connection with the analogy of furrow formation and muscle contraction. We have now examined the effects of ATP on *Limnæa* embryos and, interestingly, we have evidence for arrestation of cleavage by ATP in this material.

Limnæa eggs and embryos were collected from the underside of aquatic leaves in the local pond or from earthen vessels in the laboratory where also eggs are laid if leaves are provided. One part of the egg mass was kept as the control and the other part was treated in ATP and then put back to water. By means of such experiments we have established that ATP (100 γ/c.c.) permits cleavage but higher concentrations (1000 γ/c.c. and 500 γ/c.c.) arrest cleavage irreversibly. Some batches of eggs are arrested within half-an-hour of treatment but others require as long as one hour. Uncleaved eggs treated for one hour do not cleave at all. The ATP-arrested eggs do not show any signs of decay and degeneration that usually follow death, till two or three days later.

The inhibitory action is less in later stages of development. For example, treated trochophores, when they do not succumb to the toxic action and degenerate altogether, are not arrested, though growth rate is subnormal.

Eggs were also treated with 0·5 c.c. apyrase (activity 2·7 units/c.c.) in Tris buffer medium. These eggs stop cleaving but the inhibition is reversible if treatment is not too long.

ATP-arrested eggs (uncleaved, 2-cell, 4-cell) were treated with apyrase for varying periods and some were left in the solution but none cleaved any further.

The result with apyrase shows that it can pass through the capsule and penetrate the embryonic cells where it exerts its effect, presumably by destroying the natural ATP content of the egg. This method may perhaps be employed to investigate the biosynthesis of ATP in developing eggs. It is also clear that arrestation of cleavage is not due to the formation of a protein-ATP complex at the cell surface of such a nature that apyrase can attack the ATP. If the contractile fibre theory be correct, ATP has of course been split and the result certainly does not contradict the theory.

ATP has however a chelating action and Falk[4] offered a startling suggestion that the contraction of muscle cell by ATP is also due to chelation. We therefore compared the action of ATP with that of EDTA, a well-known chelating agent. EDTA (1500 γ/c.c.) treatment for 1 hour stops cleavage. A longer treatment is necessary at 750 γ/c.c. Some of the uncleaved, EDTA-treated eggs later underwent an alteration of shape (i.e., from spherical to dumbell) which was a clear indication of the attempt at cleavage though none succeeded in accomplishing it. Treated at 2-cell stage, some eggs may attain an "incomplete" division into 4 cells.

We are grateful to the Sigma Chemical Company for a gift of apyrase.

Unit of Embryology, Indian Statistical Inst., Calcutta-35, *May* 18, 1968.

R. L. BRAHMACHARY.
T. K. BASU.
K P. BANERJI.

1. Brachet, J., *The Biochemistry of Development*, Pergamon Press, 1960, p. 87.
2. Wolpert, L.; *Intern. Rev. Cytol.*, 1960, **10**, 207.
3. —, *Nature*, 1958, **181**, 716.
4. Falk, G., *Science*, 1956, **123**, 632.