11803

Effects of Colchicine Upon Viscosity of the Arbacia Egg.

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The work of Dustin[1] and Lits[2] on the effects of colchicine upon mammalian tissues has stimulated a large number of investigations of the cytological and cyto-genetic effects of this drug on plant and animal material. Nearly all of these studies have demonstrated that colchicine disturbs the normal processes of cell division. In contrast to the many morphological studies relatively little attention has been given to the basic physiological and protoplasmic changes produced by colchicine, changes which must be associated with the characteristic morphological alterations of the mitotic cycle. It was with the purpose of understanding further the action of colchicine on protoplasm that a study of the effects of this alkaloid on the protoplasmic viscosity of the unfertilized and fertilized egg of *Arbacia punctulata* was undertaken.

Methods. Eggs were placed in glass tubes containing 0.73 M sucrose to prevent the eggs from being thrown against the end of the tube and centrifuged in an Emerson hand centrifuge at approximately 2050 times gravity. The viscosity is expressed as the number of seconds of centrifuging necessary to stratify the eggs so that at least 80% but less than 100% showed a hyaline zone whose thickness was approximately one-sixth the diameter of the egg as measured from the periphery.[3] The centrifuging required to attain the end-point in the fertilized colchicine-treated eggs failed to throw down a considerable portion of the cortical pigment, although much of this could be moved by longer centrifuging.

The temperature varied from 21.0 to 25.3 degrees during the course of experimentation, but the variation during any single experiment was less than 2.0 degrees. Since all the experiments were not carried out at the same temperature, at any arbitrary time following fertilization different batches of eggs were in slightly different stages of development. Only batches of eggs which showed more than 95% fertilization were used.

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1 Dustin, A. P., *Bull. Acad. Roy. de Med. de Belg.*, 1934, **14**, 487.

2 Lits, F., *Comp. Rend. de la Soc. de Biol.*, 1934, **115**, 1421.

3 Angerer, C. A., *J. Cell. and Comp. Physiol.*, 1937, **10**, 183.

Colchicine in a concentration of 10^{-3} (*i.e.*, 1.0 g to 1000 cc of sea water) was used in experiments with unfertilized eggs and 0.5 x 10^{-3} for experiments with fertilized eggs. Solutions were made up fresh daily. A concentration of 0.5 x 10^{-3} is sufficiently strong to prevent mitosis from progressing further than the metaphase stage.[4] Fertilized eggs placed in this solution for as short a time as 15 minutes fail to cleave when returned to sea water. In agreement with Nebel it was found that colchicine in a concentration of 10^{-5} will not completely inhibit the cleavage of eggs placed in this solution 5 minutes after fertilization.

Results. Unfertilized eggs. The protoplasmic viscosity of unfertilized eggs immersed in 10^{-3} colchicine is not significantly different from that of eggs in sea water. The viscosity was tested over a period of 3 hours or longer on each of 7 samples of eggs. That the eggs had been affected, however, was demonstrated by immersing eggs for one hour in 10^{-3} colchicine, washing a single drop of these eggs for an hour in 2 changes of sea water of approximately 250 cc each and then fertilizing them. Fertilization membranes were formed, but in 7 experiments not a single egg was seen to have cleaved 4 hours after fertilization. Many of these eggs showed 2 light areas, which may represent daughter nuclei. (Compare Beams and Evans.[5])

Fertilized eggs. Eggs were fertilized in sea water and transferred to 0.5 x 10^{-3} colchicine 5 minutes later. The viscosity was then studied for 2 hours following fertilization.* If one determines the viscosity of normal eggs at various times before first cleavage, the viscosity will be found to increase, then decrease and be relatively low at the amphiaster stage, and finally to increase again before cleavage. (For references see Heilbrunn[6]) And in Table I the viscosity in sea water can be seen to be high 15 minutes after fertilization and to be somewhat lower 30 minutes after fertilization. However, the colchicine-treated eggs do not follow these characteristic viscosity changes. The large increase which normally follows fertilization does not take place in colchicine; and 15 minutes after fertilization the viscosity of the fertilized egg is about the same as

[4] Nebel, B. R., *Biol. Bull.*, 1937, **73**, 351.

[5] Beams, H. W., and Evans, T. C., *Biol. Bull.*, 1940, **79**, 188.

* To obviate aging of the eggs determinations for all of the intervals given in Table I were never carried out on any one sample of eggs since this would require many hours.

[6] Heilbrunn, L. V., *The Colloid Chemistry of Protoplasm*, Berlin, 1928 Chap. XV.

TABLE I.
Relative Viscosity of Eggs treated with 0.5 × 10–3 Colchicine.

Exp. No.	Unfert. eggs	15 min after fert.		23 min after fert.		30 min after fert.		60 min after fert.	120 min after fert.
		Sea water	Colch.	Sea water	Colch.	Sea water	Colch.	Colch.	Colch.
1.	60	200	70			180	80		
2.	80	190	70			160	110		
3.	60	160	80			120	100		
4.	70	180	60			140	60		
5.	60	200	70			120	100		
6.	40	200	60			160	80		
7.	60-70	180	60			120	100		
8.	60			120	60			80	120
9.	80			180	80			90	140
10.	60			200	70			70	100
11.	50			120	90			120	120
12.	60			140	60			80	70
13.	50			160	90			80	90
14.	40			180	60			100	120

that of the untreated unfertilized egg. The viscosity was found to be somewhat higher 30 minutes after fertilization in 6 of 7 cases, but a high value is not reached even after 2 hours. Beams and Evans[5] found that *Arbacia* eggs placed in 0.0002 M colchicine 10 minutes after fertilization for a period of 10 minutes are stratified more easily than eggs which have developed for 10 minutes in sea water. This result also indicates a lower viscosity for the eggs in colchicine.

The appearance of the "streak" so characteristic as a stage before the amphiaster—and one undoubtedly associated with spindle formation—is absent in colchicine-treated eggs. This is in agreement with the well-known effect of colchicine in inhibiting the formation of the achromatic apparatus.

The fusion of the pronuclei and the breakdown of the zygote nucleus are slowed in eggs whose viscosity has been kept low by colchicine. Eggs were fertilized and 5 minutes later some of the eggs were transferred to 0.5×10^{-3} colchicine. When the eggs in sea water were seen to have reached the amphiaster stage both the eggs in sea water and those in colchicine were centrifuged at approximately 2050 times gravity for 5 minutes. Most of the colchicine-treated eggs showed one or two light areas at the centripetal pole, indicating that the pronuclei had not fused (eggs with two light areas) or that they had fused but that the nuclear membrane had not as yet broken down (eggs with a single light area). Apparently colchicine has not completely inhibited the eggs in these stages, for if the eggs are centrifuged after 2 hours or longer following fertilization, the light areas are not seen, indicating that fusion of pronuclei and nuclear breakdown have occurred.

Dustin and his collaborators[7,8] believe that colchicine initiates mitosis as well as arresting the process once it has begun. Others do not agree that the number of cells in mitosis is increased by the action of colchicine.[9–12] The eggs of *Nereis limbata* were examined for mitotic activity as indicated by germinal vesicle breakdown after 3¾ to 5 hours in the following concentrations of colchicine: 10^{-4},

[7] Dustin, A. P., Havas, L., and Lits, F., *Comp. Rend. Assn. des Anat.*, 1937, **32**, 177.

[8] Dustin, A. P., *Comp. Rend. Assn. des Anat.*, 1938, **33**, 205.

[9] Ludford, R. J., *Arch. f. exp. Zellforsch.*, 1936, **18**, 411.

[10] Brues, A. M., *J. Physiol.*, 1936, **86**, 63P.

[11] Mangenot, G., *Comp. Rend. de la Soc. de Biol.*, 1938, **128**, 501.

[12] Gavaudan, P., and Gavaudan, N., *Comp. Rend. de la Soc. de Biol.*, 1938, **128**, 714.

10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10}. Seven series of experiments in which each of these concentrations was employed were carried out, and in no case was there any germinal vesicle breakdown.

Discussion. Apparently certain processes which are initiated following sperm entry and which result in increased viscosity are inhibited by colchicine. In the unfertilized egg these processes may be absent, with the result that there is no alteration of viscosity by colchicine. That is, colchicine seems to have no primary viscosity effect on protoplasm, as shown by its failure to change the viscosity of the unfertilized egg appreciably, but acts by inhibiting processes which do produce viscosity changes. Quite probably the failure of the spindle to form in the presence of colchicine is in some way related to this inhibition. The dependence of spindle formation on an increase in viscosity was expressed by Heilbrunn,[13] who found that substances which prevented an increase following fertilization also prevented formation of the spindle. Beams and Evans[5] have suggested the same for colchicine. The biochemical effects of colchicine are too incompletely known to permit any statement of the probable nature of the inhibition, but perhaps enzymatic changes are involved. The increase in viscosity which takes place prior to cleavage might also be inhibited.

Mention has been made of the fact that in colchicine division processes proceed to the point of nuclear breakdown. Now normal fertilized eggs which have reached this stage have passed through a period of high viscosity. If it is true that colchicine-treated eggs with their low viscosity have passed through a stage which in the normal has a high viscosity, then, of course, the characteristic increase in viscosity of the normal egg cannot be considered indispensable to certain early phases of mitosis.

Summary. Colchicine in a concentration of 10^{-3} in sea water does not appreciably alter the viscosity of the unfertilized *Arbacia* egg. The increase in viscosity which normally follows fertilization is inhibited by colchicine in a concentration of 0.5×10^{-3}. Fusion of the pronuclei and nuclear breakdown are delayed in colchicine-treated eggs. Colchicine in concentrations of 10^{-4} to 10^{-10} does not initiate mitosis in the *Nereis* egg.

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[13] Heilbrunn, L. V., *Anat. Rec.*, 1917, **11**, 487.