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Colloidal Properties of Nucleus. I. Effect of Temperature on Nuclear Viscosity in the Starfish Egg.

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Although the colloidal properties of the nucleus are important to an understanding of its role in cell division, very little information of a quantitative nature has been obtained from the living cell. It is evident from the observations of Gray[1] and Harris[2] that the nuclear contents of certain marine eggs are in a liquid state. Gray noted that the nucleolus in the germinal vesicle of the Echinus egg moved under the influence of gravity, and he determined the rate of its movement. Heilbrunn[3] was able to estimate the absolute viscosity by substituting the value for the rate of movement into Stokes' formula and assuming the densities of nucleoplasm and nucleolus on theoretical grounds. Harris[2] from his photographic observations on nucleolar movement calculated the absolute viscosity of the germinal vesicle of the Echinus egg to be about 10 centipoises, and he checked this value with determinations of viscosity by Pekarek's Browian movement method. He also found that a fall of the nucleolus was clearly seen in the eggs of 4 other species of echinoderms. It seems, therefore, that in the germinal vesicles of certain marine eggs, the viscosity is approximately 10 times that of water.

The purpose of this investigation is to determine the effect of temperature on nuclear viscosity in the egg of the starfish, *Asterias vulgaris*, by means of the falling nucleolus method. This method has the distinct advantage of yielding quantitative results without harming the egg in any way.

Procedure. Eggs in the immature germinal vesicle stage were used. The ovaries from freshly dissected animals were placed in sea water in fingerbowls, and the eggs, which were shed almost immediately, were kept at the temperature of running sea water (about 19°C) until used. Eggs removed from the animal for more than 3 hours were discarded. For each experiment a drop of dilute egg suspension was placed on a depression slide with a rectangular depression approximately 125 μ deep. A few strands of absorbent cotton were put in the chamber to keep the eggs in position and a coverslip lightly ringed with vaseline was placed on top. The eggs were then observed through a horizontal microscope.

After the suspension had remained a few minutes in this position, an egg was selected for observation. The microscope stage was then revolved 180° and the movement of the nucleolus through the entire diameter of the nucleus timed. In each observation the time was measured from the instant the stage was revolved until the nucleolus was observed to reach the bottom of the nucleus. This was repeated several times with each egg.

Temperature was varied by placing the microscope in a cold room to obtain temperatures between 3°C and 24°C. For higher temperatures the microscope was placed in a constant temperature box equipped with heating coils. Readings were made with the thermometer bulb directly on the stage. For each experiment the microscope was allowed to come to equilibrium with the desired temperature for 15 minutes or more.

Results. The results obtained are summarized in Table I, which shows the average number of seconds required for the nucleolus to fall at each temperature studied. When the values in the third column of Table I are plotted against temperature a straight line relation is suggested. The data were analyzed by the method of least squares. The resulting equation is:

1 Gray, J., *Brit. J. Exp. Biol.*, 1927, **5**, 102.

2 Harris, J. E., *J. Exp. Biol.*, 1939, **16**, 258.

3 Heilbrunn, L. V., *The Colloid Chemistry of Protoplasm*, Berlin, 1928.

TABLE I.
Effect of Temperature on Time of Fall of Nucleolus and the Thixotropy of the Nuclear Colloid.

Temp. °C	Time for initial fall, sec.	Avg of times for subsequent falls, sec.	Ratio initial time to avg. of subsequent times	No. of experiments	Total No. observations
3.5	285	230	1.239	1	8
8.9	235	204	1.152	1	7
10.5	273	223	1.225	3	30
12.5	220	199	1.103	3	12
14	215	158	1.360	1	10
16	200	167	1.188	6	39
17	185	153	1.294	3	22
19	165	137	1.205	3	14
23	150	142	1.056	1	5
24	144	130	1.105	7	49
25	133	122	1.097	9	58
27	122	128	0.957	8	52
28	104	112	0.941	5	51
30	95	102	0.983	1	12
33-35.5	120	81*		1	10
33-37	95	95†		1	2

* Nuclear colloid gelled after tenth reading.
† Nuclear colloid gelled after second reading.

$$Y = 245.1 - 4.865\ T$$

where Y = Time for nucleolus to fall through entire diameter of nucleus (seconds).
T = Temperature (degrees C).

The line defined by this equation is shown in Fig. 1, the plotted points representing averages of all determinations for each 5 degree interval. Since viscosity varies directly with the time, corresponding values for viscosity were included on the ordinate scale. These values were calculated from Stokes' formula by substituting 7.17×10^{-4} cm for nucleolar radius, 56.6×10^{-4} cm for the distance the nucleolus falls, and 0.1 for the difference in density between nucleoplasm and nucleolus. The latter value for difference in density is theoretical. Justification for this is given by Heilbrunn[3] in his monograph.

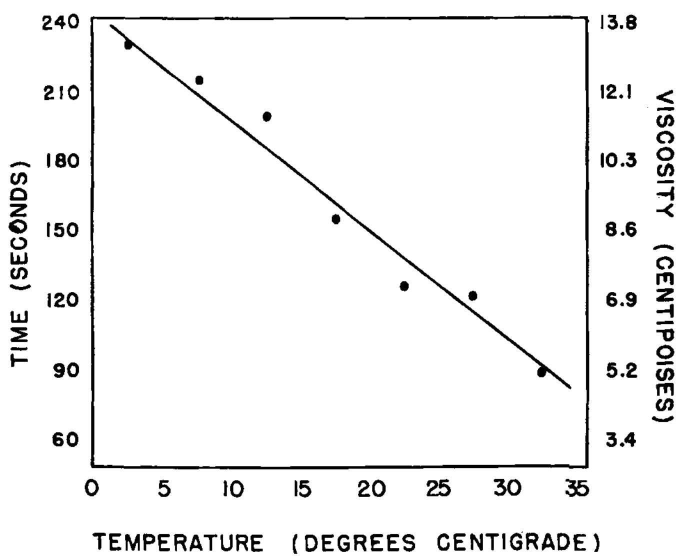

FIG. 1.
The effect of temperature on the time of fall of the nucleolus. Corresponding values for viscosity are on the right side of the graph.

TABLE II.
Results of Application of the t-test in Determining Significance of Deviations of Mean Differences from the Theoretical Value of Zero for Two Temperature Intervals.

	Temp. range (°C) 3.5-24.5	25.0-30.0
Mean value of differences for given temperature interval	28.75	—1.4
Deviation of mean value of differences from *O*	28.75	—1.4
t value	6.268	0.489
No. of experiments	28	20
Probability of obtaining such a deviation by chance alone from the theoretical mean of *O*	0.00	0.63

Ladenburg's correction factor was used. This corrects for the effect of the nuclear membrane on the velocity of the moving nucleolus. Although the equation has not been adapted for a sphere moving within a sphere, Harris from observations on a model nucleus deduced an empirical value of 0.29 for the Echinus egg nucleus. Since the relative proportions of nucleolus and nucleus are approximately the same in the Asterias egg as they are in the Echinus egg the same value was used in these calculations. As can be seen from Fig. 1, the absolute viscosity, determined in this way, is about the same as that found in the Echinus egg by Harris. It would appear, therefore, that at least in the temperature range investigated the absolute viscosity changes by approximately 2.7 centipoises with a 10°C change in temperature. This change is evident up to temperatures of approximately 35°C. In this range the nuclear contents gel, as is evident from the third column in Table I, which indicates that the nucleolus did not move at or above this temperature.

Also, from Table I, the thixotropic nature of the nuclear colloid is suggested. In the fourth column the mean ratio of initial reading to average of subsequent readings is given for each temperature employed. If the nucleoplasm is a thixotropic colloid, this ratio should be greater than one. This is found to be the case at lower temperatures. However, the values appear to decrease with increase in temperature until a ratio close to unity is reached. This is at a temperature of approximately 25°C. Apparently, therefore, temperatures of 25°C or above abolish thixotropy.

In order to test the significance of this apparent change in thixotropy the differences between the initial readings and the averages of subsequent readings were analyzed by means of the t-test (based on Student's distribution). The mean of the differences obtained in experiments at temperatures of 25°C through 30°C proves to be insignificantly different from zero. The mean of the differences corresponding to temperatures of 3.5°C to 24.5°C, however, is significantly greater than zero. The numerical results of these calculations are given in Table II.

Discussion. There were no marked deviations from a linear relationship between viscosity and temperature within the range studied. This is unlike the cytoplasmic reaction in the Cumingia egg as found by Heilbrunn.[4] In this case the viscosity goes through a maximum value at 15°C. In some other cells, however, the cytoplasmic viscosity decreases progressively as the temperature is raised until the protoplasm gels (see Heilbrunn[3] for discussion). This is similar to the results obtained with the starfish egg nucleus. Coagulation of the starfish egg nucleoplasm occurred at approximately 35°C. For the present data, the latter value is necessarily an approximation since a complete description of heat coagulation would involve not only the temperature but also the time that the eggs were exposed. Heilbrunn[5] has shown that the protoplasm of the Arbacia egg coagulates at 31°C to 37°C, that of the Cumingia egg at higher temperatures. In both cases, the temperature coefficient of the change in rate of coagulation per degree change in

4 Heilbrunn, L. V., *Am. J. Physiol.*, 1924, **68**, 645.

5 Heilbrunn, L. V., *Am. J. Physiol.*, 1924, **69**, 190.

temperature lies between 1 and 2. Investigations are planned to determine other properties of the nuclear colloid, particularly with regard to its radiosensitivity. The present knowledge of temperature effects will serve as a guide to the proper experimental conditions.

Summary. By means of the falling nucleolus method it was determined that a 10°C increase in temperature lowers nuclear viscosity in the Asterias egg by approximately 2.7 centipoises. Thixotropic properties disappear at temperatures above 25°C and coagulation occurs at about 35°C.

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Effect of Stretch and Pressure on Stimulus Formation in the Dog's Auricle.

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The application of a few crystals of aconitine or of 0.05 cc of a 0.05% solution of aconitine to the auricular appendix of the exposed heart of the dog results in a regular tachycardia with a rate of 300-500 beats per minute. Invariably this tachycardia responds to faradic stimulation of the vagus nerves with an increase of rate.[1] The administration of aconitine to the site of the sinus node also leads to a tachycardia which spontaneously or following stimulation of the vagus or sympathetic nerves is transformed into auricular fibrillation.[2,3] Because of its high rate and the ease of its transformation into fibrillation the auricular tachycardia was considerd to be auricular flutter. Cooling the small area to which the aconitine has been applied stops the fibrillation (or flutter); it reappears immediately when cooling is interrupted.[2] Earlier it was pointed out that these observations are not compatible with the assumption that auricular fibrillation is due to a circus movement mechanism. An extremely rapid stimulus formation with secondary formation of reentry waves was considered the responsible mechanism.[2]

This report deals with observations made during application of stretch and pressure on the right auricle of the dog's heart in the presence of an aconitine-induced tachycardia.

Method. The technic employed was the same as in previous investigations. The heart was exposed with the dog under nembutal anesthesia and artificial respiration. Aconitine was injected into the region of the head of the sinus node. All electrocardiograms were recorded in lead II. Stretching of the auricle was accomplished by one of several methods: 1) by attaching a weighted hook to the right auricular appendix or the right auricle at the junction between the vena cava superior and the right appendix; 2) by stretching the tip of the appendix of the right auricle by means of a blunt forceps; 3) by rapid intravenous infusion of 50 cc of 0.9% saline at body temperature into the jugular vein or the vena cava superior. Pressure on the area into which aconitine had been injected was exerted by a probe. In all experiments we satisfied ourselves that the site of application of aconitine actually was the focus of stimulus formation because cooling of this site invariably resulted in a cessation of flutter or fibrillation as long as cooling continued.

Results. In 11 experiments stretching led to an increase of auricular rate and to auricular fibrillation. The fibrillation sometimes developed so quickly that the transitional period of tachycardia could not be analyzed. More often a short period of increased rate preceded the fibrillation. The duration of the

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1 Scherf, D., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 233.

2 Scherf, D., Romano, F. J., and Terranova, R., *Am. Heart J.*, 1948, **36**, 241.

3 Scherf, D., *Am. Heart J.*, in press.