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The Significance of Gelation in the Systole of the Contractile Vacuole of *Amoeba dubia*.

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(Introduced by Robert Chambers.)

From Washington Square College and Cornell University Medical College.

I. MECHANICAL SUPPORT DURING SYSTOLE.

Observations of the position and behavior of the contractile vacuole of *Amoeba dubia*, lead to the conclusion that, under normal conditions, the vacuole, at systole, is lodged in a region, which, judged by certain optical and experimental criteria, is gelled. The optical evidences of this gelation are: first, under ordinary transmitted illumination, the absence of translatory movement, and the relative fixed position of the granules and crystals involved; second, on dark field examination, the absence of Brownian movement, and the appearance of fibrillar lines in this region. The experimental evidence is derived from: first, testing the consistency of the endoplasm by means of a microneedle; second, repeated local agitation of an endoplasmic region, which causes it to change from a motionless (and apparently gelled) to a distinctly flowing state. This last is interpreted as due to a reversal of phases.

The constant occurrence of this encapsulating area of gelation in the normal ameba, suggests that it functions as a source of mechanical support for the vacuolar membrane during systole. It is not unreasonable to assume that a phenomenon analogous to syneresis in the gelled region may initiate systole. Experimental evidence confirms the first of these assumptions. The microinjection of urea* greatly increases the fluidity of the internal protoplasm, so that the ameba takes on a limax form. The lack of gelation results in the absence of internal mechanical support for the vacuole, which becomes greatly dilated.

II. EFFECT OF INJECTED WATER.

In accordance with the results of Chambers and Reznikoff,¹ the gradual injection of distilled water at a pH of approximately 6.6 to 6.8, affects the physiological state of the organism but slightly. The ameba retains its normal irregularities of form, but we find that the rate of contraction of the vacuole is accelerated. Comparisons of the fluid output of a single vacuole before and after injection,

* To be reported in a separate paper.

show a definite increase in vacuolar output subsequent to injection.

Rapid injection of a large quantity of water produces a condition which apparently requires profound physiological reorganization. After the first violent reaction to this treatment, the ameba temporarily assumes a limax form, and, during the readjustment period the diastolic interval of the vacuole is lengthened. As the organism approaches the normal state, gelled regions reappear, and the vacuole shows a marked increase in the fluid output. Repeated small injections, in close sequence, may result in the production of a fluid limax form, which then becomes readjusted to the normal state in the manner just described for large injections. During readjustment, the rate of contraction is accelerated and the fluid output is greatly augmented.

¹ Chambers, Robert, and Reznikoff, Paul, *J. Gen. Physiol.*, 1926, viii, 369-401.

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Microinjection of Urea into the Protoplasm of *Amoeba dubia*.

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Urea is slowly but progressively toxic to amebae when added to the immersing fluid. This toxic condition is evidenced by withdrawal of radiate pseudopods, change to unipodal form of locomotion, and, finally, by rounding up and quiescence. If sedimentation of granules and crystals is taken as a criterion of fluidity, it is concluded that the endoplasm is in a state of liquefaction in a high percentage of cases (M/12 to M/48 urea).

Injection of urea into the internal protoplasm uniformly produces liquefaction. The contractile vacuole, thus deprived of a gelled region for mechanical support,* becomes greatly dilated. It is usually carried anteriorly, and may be artificially emptied by pressure of a blunt microneedle. Small accessory vacuoles of the explosive type may still function in the gelled area maintained just under the plasmalemma. After injections of strong solutions, the plasmalemma blisters over the entire surface. Dilute injections cause the formation of clusters of uroid-like pseudopods.

* See previous paper in same issue.