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## Fate of the Crystals in Amebas.

By

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The crystals and crystalline granules in ciliates and rhizopods have generally been regarded as excretion products. This view follows from a consideration of their supposed chemical identity with the salts of uric acid, calcium oxalate, calcium phosphate, and other excretion products of plants and animals, and from the early observations of F. STEIN and others. STEIN (1859) recorded having observed that the crystalline granules of *Paramecium bursaria* were eliminated with excrement. But neither MAUPAS (1883) nor SCHEWIAKOFF (1893), were ever able to observe that this took place in *P. aurelia* or in *P. caudatum*, respectively.

SCHEWIAKOFF observed that the crystals gathered in the region of the contractile vacuole. There, they broke into small pieces, dissolved, and were eliminated, presumably, with the fluid of the contractile vacuole, this being in accord with the view of ENTZ (1879). Other views were held by RHUMBLER (1888) and SCHAUDINN (1899). RHUMBLER observed a breaking up of the granules of *Colpoda* and the expulsion of the pieces in solid form by the contractile vacuole. In the rhizopods, SCHAUDINN observed in toto ejection of the granules of *Trichosphaerium*. SCHAEFFER (1919, 1923) suggested that the crystals of amebas are neither excreted by, nor destroyed within the ameba, but persist as long as the ameba lives. Finally.

MAST and DOYLE (1935) reported that the crystals in *Amoeba proteus* slowly dissolved within the organism.

Many of the larger amebas contain so many crystals that it is not possible to trace the changes in the number and size of the crystals over long periods of time. In order to obviate this difficulty, I have studied a small bacterium-eating ameba, *Cochliopodium bilimbosum*, specimens of which may contain so few as six or seven crystals. Isolated amebas were studied in hanging drop preparations in which they fed and reproduced for a period of weeks.

The crystals in *Cochliopodium bilimbosum* are identical in physical and chemical properties with the bipyramidal crystals of *Chaos diffluens* (*Amoeba proteus*) and *Trichamoeba villosa*.

*Cochliopodium* excretes its crystals in toto. This, however, is of relatively rare occurrence, for it is possible to watch for as long as twelve hours without observing it. I have seen it happen five or six times. The actively feeding amebas are continually forming excretion vacuoles which increase in diameter to about seven microns. The vacuole moves to the posterior edge or to the side of the ameba, where it forcibly ejects its excrementitious contents. This takes place quite frequently, but in the majority of times, no crystals are expelled. When a crystal is to be expelled, it usually is the largest in the ameba. It moves into the excretion vacuole, and with excrement, is pushed out into the surrounding medium where it dissolves within a few minutes.

The following is a record of the number and size of crystals in an ameba and its two fission products, one of which expelled a crystal:

	Length	Breadth	Thickness
3-22-37 10 am.	4.1 $\mu$	2.9 $\mu$	1.8 $\mu$
	3.4	2.6	1.5
	3.4	2.5	1.5
	3.4	2.5	1.4
	3.4	2.4	1.5
	3.3	2.4	1.5
	3.3	2.5	1.4
	3.1	2.4	1.3
	2.7	2.0	1.2
	2.7	1.9	1.1
	2.7	1.9	1.2
	2.6	2.0	1.1
	2.6	1.9	1.0
	2.0	1.5	1.0
	nine crystals less than 2 microns long		

	Length	Breadth	Thickness
First Fission-product	4.8 $\mu$	3.4 $\mu$	2.0 $\mu$
3—22—37 7 pm.	3.4	2.7	1.4
	3.4	2.7	1.4
	3.4	2.6	1.3
	3.3	2.6	1.3
	3.3	2.5	1.2
	3.2	2.4	1.2
	2.7	2.1	1.0
	1.4	1.0	0.7
	1.4	1.0	0.6
	1.3	1.0	0.6
	1.3	1.0	0.6
	three crystals less than 1.3 microns long		
Second Fission-product	4.1	3.3	1.8
3—22—37 7 pm.	4.0	3.0	1.7
	3.5	2.2	1.3
	3.3	2.3	1.5
	3.3	2.2	1.3
	3.3	2.3	1.2
	eleven crystals less than 1.4 microns long		
Second Fission-product	4.6	3.5	1.6
3—23—37 7 am.	4.1	2.8	1.5 (This crystal expelled)
	4.1	2.8	1.5
	2.8	2.2	1.3
	2.6	2.1	1.1
	2.5	2.2	1.1
	2.4	2.2	1.0
	nineteen crystals less than 2.0 microns long.		

Studies were also made on abnormal examples of *Cochliopodium*, that is, on individuals which neither fed, reproduced, nor moved in a coordinated manner. Two additional symptoms of a pathological condition are observable in such amebas: (1) More crystals are expelled than normally, and (2) no new crystals are formed. After death, the crystals decrease in size, but in abnormal as well as in normal amebas, the crystals increase in size until they are expelled.

It remains to be stated that since excretion of the crystals of at least one ameba is a relatively rare occurrence, the crystals are kept from accumulating in large numbers, not by being excreted, but by virtue of their being approximately halved each time the organism undergoes fission.

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