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# LXVII. THE INFLUENCE OF WASHING UPON THE REPRODUCTIVE RATE OF *COLPIDIUM COLPODA*.

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IN a recent paper Robertson [1924, 1] reports that he has observed allelo-catalysis in cultures of *Colpidium* sp. both in hay infusions and in synthetic medium. He also explains our earlier failure to obtain this effect in cultures of *Colpidium colpoda* [Cutler and Crump, 1923] as being due to too great a concentration of the parent culture medium in the sub-cultures (a dilution of from 1/37–1/980), and claims that if the organisms had been washed until the dilution was considerably greater (1/9000 in his own case) the allelo-catalytic effect would have been observed.

This is of course a direct contraversion of certain facts published by this author in 1923 [Robertson, 1923, p. 97], which demonstrated that the allelo-catalytic effect occurs in sub-cultures in which large quantities of "auto-catalyst" derived from the parent must have been present. "Into a drop, measuring about 0.08 cc. of freshly prepared hay infusion was isolated an infusorian (*Enchelys*) derived from a densely inhabited parent-culture which was 7 days old. At the same time two separately isolated individuals from the same parent-culture were introduced together into a similar drop of infusion. The following results were obtained:"

Culture number	Number of individuals initially introduced	Number of individuals	
		After 24 hours	After 48 hours
310 A	1	2	16
311 A	2	3	120

Here, if we may take it that the individuals were isolated into drops of a similar size to those used in other experiments, namely, 0.002–0.003 cc., the dilution is only 1 in 40, and moreover the parent culture had been the scene of very active reproduction.

Under similar conditions we have demonstrated that there is no trace of mutual acceleration in individuals of *Colpidium colpoda*, as is shown in Table I, compiled from the data given in a previous paper [Cutler and Crump, 1923]. The cells in question were isolated from a 2 days old culture in which there had been rapid reproduction and they were grown, in the usual way, in chambers containing synthetic medium.

Table I. *Reproductive rate after 48 hours in cultures in which the dilution of the parent culture liquid in the sub-culture is less than 1 in 200.*

	Reproductive rates	Average
One animal	2.0, 2.0, 5.1, 3.8	3.2
Two animals	3.6, 1.0, 2.4, 3.5	2.6

The present paper gives the results of a series of experiments on *Colpidium colpoda* in which the organisms were washed before isolation thereby reducing the concentration of autocatalyst carried over from the parent culture to an amount comparable with that in Robertson's experiments. The washing was effected by centrifuging a culture of known ancestry, *i.e.* age of culture and amount of reproduction which had occurred, siphoning off the supernatant liquid, and adding fresh medium to the remaining 1 cc., and repeating this process until a suitable dilution was reached. From the final liquid the animals were isolated into a capillary tube with about 0.2 mm.<sup>3</sup> of fluid, and inoculated into a chamber containing a quantity of medium varying from 1.6–9.2 mm.<sup>3</sup>, in which bacteria from a 1 day old agar slope had been emulsified. The dilution of the parent culture varies in these experiments from 1 in 2540 to 1 in 22,900.

It is necessary to point out that where a small quantity of liquid containing rapidly moving organisms is delivered from a fine capillary tube it does not follow that the same number of organisms will always be contained in every drop, hence one or two organisms from the same parent culture can be obtained in drops whose sizes vary only within very narrow limits.

Table II shows the results obtained in sub-cultures from 1 and 2 animals which have been washed before inoculation.

Table II. *Reproductive rate after 24 hours' growth of washed individuals derived from young parent cultures (24–48 hours old).*

Number originally isolated	Degree of dilution of fluid from parent culture in liquid of sub-culture		
	1 in 1,000–5,000	1 in 5,000–10,000	1 in 10,000–20,000
1	4.0, 4.1, 3.3, 4.1, 4.7, 3.3, 3.0	4.8, 3.0, 3.0, 3.0, 4.0, 3.0, 4.0, 4.0, 4.1, 4.6, 4.0	3.5, 3.3, 4.2, 3.8, 4.0, 4.0, 4.0, 4.0, 4.6, 4.0, 4.0, 4.1, 4.2, 4.6
Average	3.8	3.8	4.2
2	3.9, 3.8, 4.2, 4.2, 3.3, 3.9, 2.4, 3.6	3.3, 2.5, 3.0, 4.0, 3.3, 2.0, 2.9, 4.0, 3.9	3.5, 3.9, 3.0, 4.3, 4.2, 3.9, 4.2, 3.7, 3.8, 3.9, 3.7, 3.2, 3.9
Average	3.7	3.2	3.8

After 48 hours' growth the averages for the same series of cultures are:

One animal	7.0	6.6	7.4
Two animals	5.6	6.1	6.6

It is obvious from the figures that in *Colpidium colpoda* allelocatalysis does not occur under the conditions in which Robertson has detected it both in *Enchelys* and in the other species of ciliate which he doubtfully assigns to the genus *Colpidium*. His description and figures of the animal, however

[Robertson, 1924, 2], indicate that he was not dealing with any species of the genus *Colpidium*, but more probably with one of the *Colpoda* genus.

The fact that this series of experiments shows higher reproductive rates at 24 hours than those given in our earlier paper [Cutler and Crump, 1923, 2], e.g. 3.8 as against 2.4, is due to the fact that in this series the cultures were fed with an unidentified species of bacterium [Cutler and Crump, 1924] which gives uniformly better results than are obtained where the cultures are treated with *Sarcina* as was the case in the earlier experiments.

To support his contention Robertson has also re-grouped some of the figures given in our earlier paper [Cutler and Crump, 1924] on *Colpidium colpoda*, and from these derives further support for his theory that the reproductive rate is retarded by a high concentration of the substances introduced with the organisms from the parent culture, because retardation appears to occur in the cultures of smaller volume into which the individuals were introduced. Column 1 of Table III shows the figures quoted by Robertson; the other columns he omitted in his paper. The numbers given are, however, all averages of individual reproductive rates and statistical treatment shows that the variation between the individual reproductive rates in any one group is as great as it is between those of different groups; and that therefore the apparent rise in the average reproductive rate from the smaller to the larger drops is a matter of pure chance; in short, there is no significant difference between any of the numbers in the original table.

Table III.

Vol. of substance in cu. mm.	Average reproductive rate in first 24 hours			
	One animal	Two animals	Three animals	Four animals
0-1.5	1.98	2.49	2.39	2.31
1.5-3.0	2.27	2.29	2.36	2.46
3.0-6.0	2.86	3.75*	2.46	2.50
6.0-9.0	2.87	2.84	(No cases)	3.30*

\* Only two cases.

A further difference between the results obtained by Robertson and ourselves lies in the effect of washing on the organisms. Robertson [1924, 2] records that in his *Colpidium* sp. abnormal forms were sometimes produced in cultures from washed individuals, but in the case of our organism abnormal animals have never been seen. He has further found that washing affects the division of his species in that the animals in cultures from unwashed individuals tend to divide into four, those from individuals that have been washed once into either two or four, and those from individuals washed two or three times into two only. Again in the case of *Colpidium colpoda* this difficulty in the study of reproductive rates does not occur since reproduction in this species is by binary fission only, and washing does not retard the rate of reproduction as is shown in Table IV.

Table IV. *Average reproductive rates in cultures from washed and unwashed individuals.*

Initial inoculum	Unwashed.	Washed.	
	Dilution of parent liquid less than 1 in 35	Dilution of parent liquid	
		1/5,000–1/10,000	1/10,000–1/200
One animal (after 24 hours)	4.45	3.75	3.86
Two animals (after 24 hours)	3.10	3.55	3.67
One animal (after 48 hours)	7.35	6.67	7.52
Two animals (after 48 hours)	6.75	5.70	6.55

# SUMMARY.

In *Colpidium colpoda* allelocatalysis has not been observed in cultures either from washed or unwashed individuals.

Washing the animals has no deleterious effect upon them nor does it retard the reproductive rate.

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