THE TRANSFORMATION OF BRACHIONUS PALA INTO BRACHIONUS AMPHICEROS BY SODIUM SILICATE.

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In certain species of rotifers there is considerable variation in the shape and form of the body of the female during the year. Powers has shown that in one species of *Asplanchna* there are three distinct phases through which a race may pass during one season. The females in each phase differing from those in either of the other two phases in both size and general form of the body. He and Mitchell also have shown that in this particular species these changes are somewhat corellated with the sexual stage of the females which is produced by changes in food. Sachse studied the variability of form in several other species and found that the greatest variability occurred at the periods of abundant food but he did not find that this variability was in any way correlated with the sexual stage. Lauterborn made observations upon *Anuraea cochlearis* and concluded that the variations of form were due to changes in temperature. Wesenberg-Lund made observations upon *Asplanchna priodonta* and concluded that the variations were due to differences in density of the water which varied according to the temperature.

Recently H. K. Harring, custodian of the Rotatoria in the United States National Museum, pointed out to me that *Branchionus amphiceros* was a variation of *Branchionus pala* and that it probably could be artificially produced from *Branchionus pala* at any time by changes in diets. Knowing, however, only one diet on which this species would thrive readily in the laboratory it was impossible to test this suggestion.

In considering the problem the self-evident fact was recalled that the young stages of all living organisms must be supplied with suitable materials out of which to build the various tissues of their bodies. If an animal has a lime or a siliceous skeleton it must be supplied with calcium or siliceous salts with which to

build its framework. In all rotifers there is some skeletogenous tissue and in most species the greater part of it forms the external covering. In some species this external skeleton is excessively delicate and flexible while in other species it is relatively thick and non-flexible. This external covering is usually considered as being composed of a chitinous material. In general the different species of rotifers are distinguished from each other by the different forms of this external covering. If one could furnish an abundance of material such as the animals use in making this covering it would seem that there might be an opportunity for obtaining variations in the form and size of the covering. Not knowing how to obtain chitin in a liquid form other materials were considered. Sodium silicate (water glass) was used first and was at once successful beyond all expectations.

Brachionus pala possesses two small posterior spines, one on each side of the base of the tail, whereas the variation *Brachionus amphiceros* not only possesses these two spines which are usually longer but also possesses in addition two large lateral posterior spines. These lateral spines vary somewhat in size in different individuals and in the same individual they are much larger in proportion to the size of the body in the young stage than in the adult stage.

During the spring and summer both forms are found but there is considerable irregularity in their appearance. In the spring *Brachionus pala* may be found almost exclusively, while later *Brachionus pala* and *Brachionus amphiceros* may occur in equal numbers and still later, from June to October, *Brachionus amphiceros* may form as high as 93 per cent. of the total collections of the two forms, as was found by Kofoid. The exact cause of the fluctuating appearance of these two forms in the same body of water is not known.

In the first series of experiments one drop, two drops, three drops, four drops, and five drops of sodium silicate were added respectively to five jars each containing 150 c.c. of beef bouillon culture medium. These jars were inoculated with pure cultures of the green flagellate, *Chlamydomonas*. As soon as there was a vigorous growth of the *Chlamydomonas* several dozen females of *Brachionus pala* were put into each jar. After a few days had

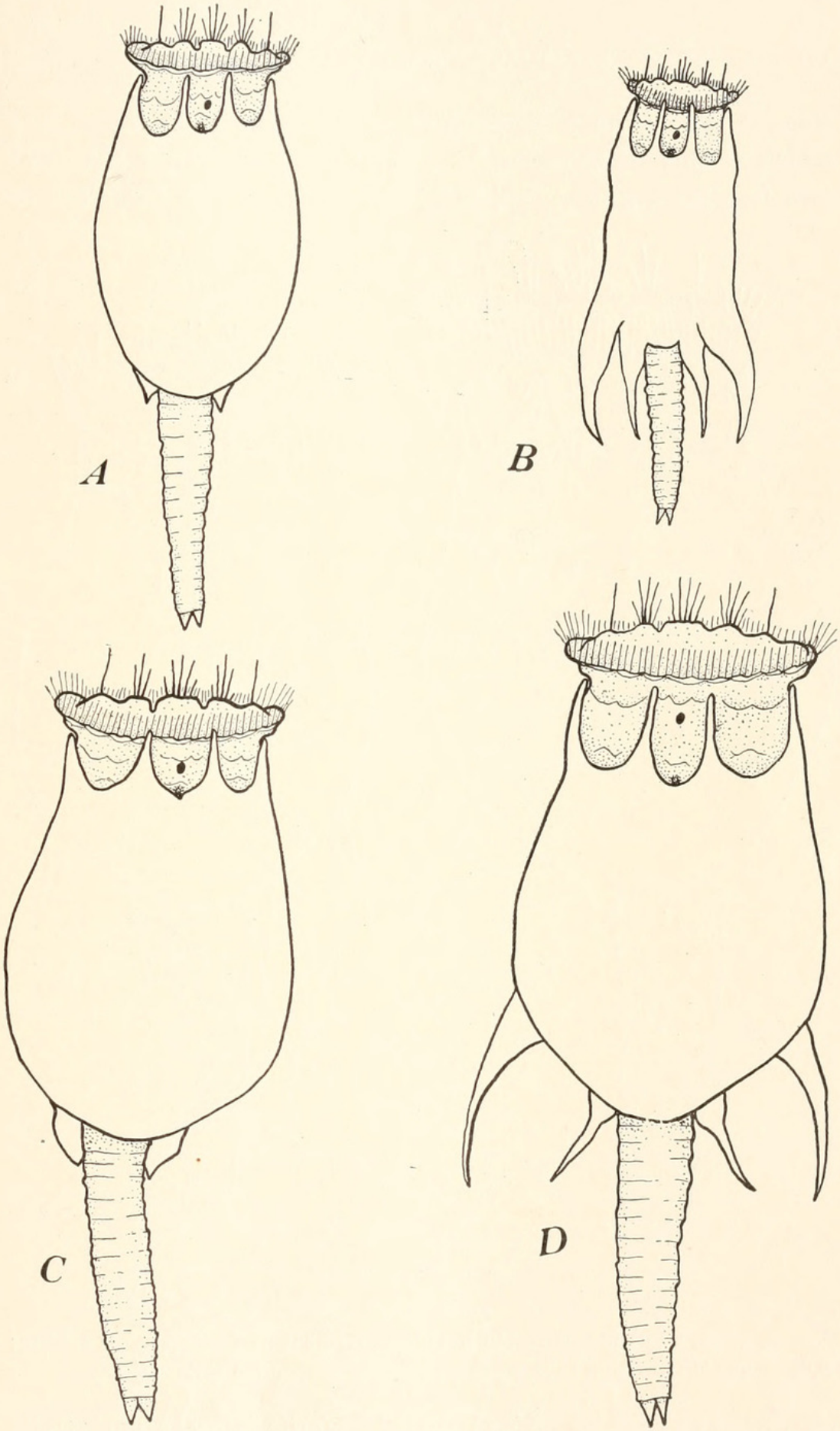

FIG. 1. *A*, young female just after emerging from a parthenogenetic egg which was produced in culture medium free from sodium silicate; *B*, young female just after emerging from a parthenogenetic egg which was produced in a culture medium containing sodium silicate; *C*, similar to *A* only older and larger; *D*, similar to *B* only older and larger.

elapsed observations were made on the rotifers in these jars which at this time contained many thousands. In the control jars free from sodium silicate only three females from nearly

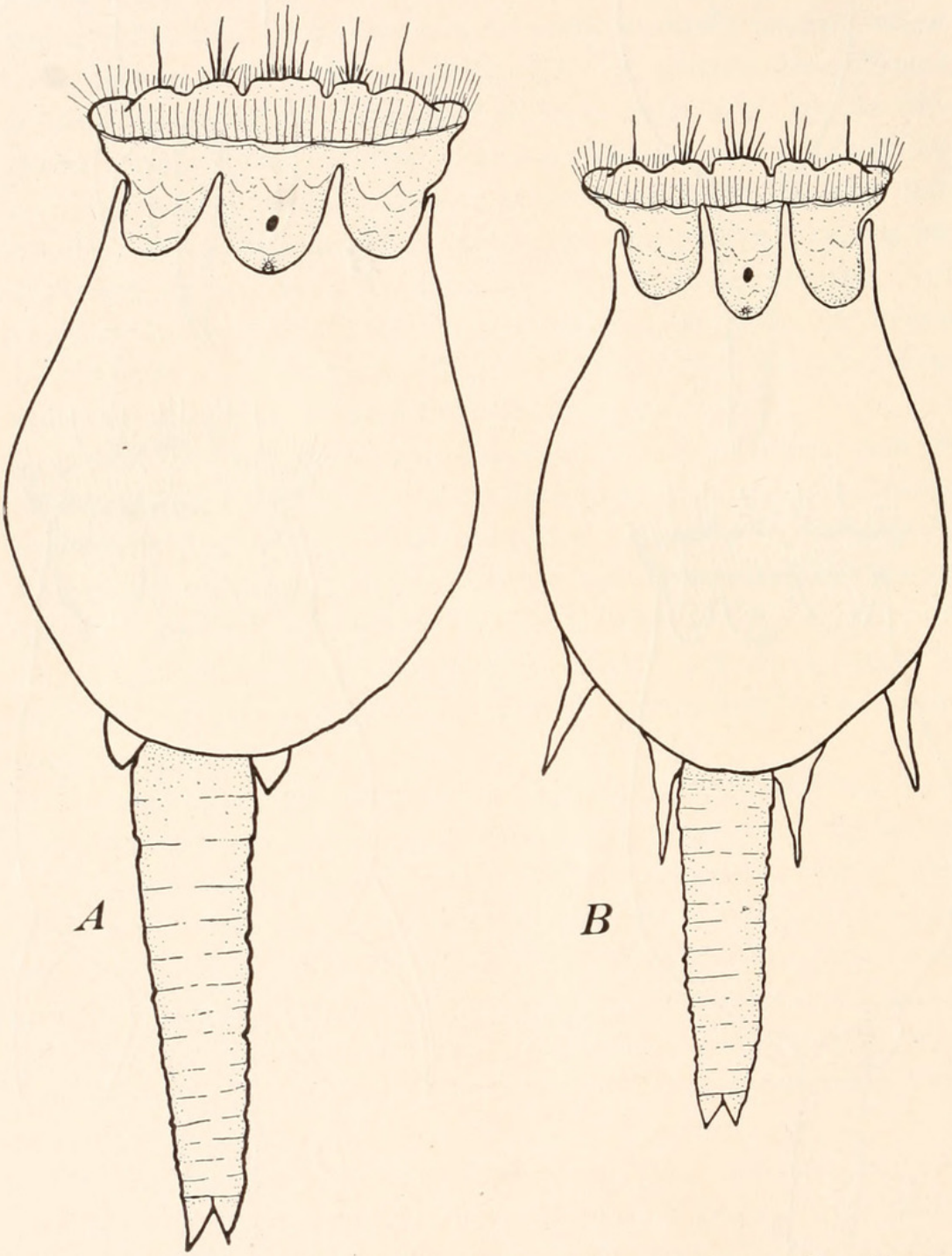

FIG. 2. *A*, adult female reared in culture medium free from sodium silicate; *B*, adult female reared in culture medium containing sodium silicate.

six thousand females possessed the posterior lateral spines while in the jars containing sodium silicate many of the females possessed the posterior lateral spines. In some of the jars containing

five drops of sodium silicate every female possessed these additional spines and in all the jars containing ten drops of sodium silicate every female also carried these spines. Not only did the

TABLE I.

SHOWING THE INFLUENCE OF SODIUM SILICATE IN THE PRODUCTION OF TWO ADDITIONAL POSTERIOR LATERAL SPINES IN THE FEMALES OF *Brachionus pala* THUS FORMING *Brachionus amphiceros.*

Experiments.	Observations, 1916.	Control Culture Medium Free from Sodium Silicate.			One Drop of Sodium Silicate in 150 c.c. Culture Medium.			Four Drops of Sodium Silicate in 150 c.c. Culture Medium.			Five Drops of Sodium Silicate in 150 c.c. Culture Medium.			Ten Drops of Sodium Silicate in 150 c.c. Culture Medium.		
		Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.
1	Jan. 27....	998	2	.002	90	10	10	4	96	96	0	50	100	...	...	...
	Feb. 2.....	1,000	0	0	88	12	12	3	97	97	0	200	100	...	...	...
	Feb. 7.....	100	0	0	...	...	...	0	100	100	0	100	100	...	...	...
	Feb. 14....	99	1	1	...	...	...	0	100	100	1	19	95	...	...	...
2	Feb. 21....	200	0	0	...	...	...	...	...	....	10	90	90	...	...	...
	Feb. 25....	500	0	0	...	...	...	...	...	....	100	400	80	0	300	100
3	Feb. 21....	100	0	0	...	...	...	...	...	....	50	50	50	0	100	100
	Feb. 25....	100	0	0	...	...	...	...	...	....	250	250	50	0	100	100
4	Feb. 21....	100	0	0	...	...	...	...	...	....	25	75	75	0	100	100
	Feb. 25....	100	0	0	...	...	...	...	...	....	150	450	75	0	100	100
5	April 13...	100	0	0	...	...	...	...	...	....	10	115	92	...	...	...
	April 16...	300	0	0	...	...	...	...	...	....	15	285	95	...	...	...
6	April 13...	200	0	0	...	...	...	...	...	....	2	20	90	...	...	...
	April 16...	250	0	0	...	...	...	...	...	....	9	291	97	...	...	...
7	April 13...	175	0	0	...	...	...	...	...	....	14	86	86	...	...	...
	April 16...	150	0	0	...	...	...	...	...	....	30	270	90	...	...	...
8	April 13...	100	0	0	...	...	...	...	...	....	13	91	87	...	...	...
	April 16...	300	0	0	...	...	...	...	...	....	30	270	90	...	...	...
9	April 13...	230	0	0	...	...	...	...	...	....	9	103	91	...	...	...
	April 16...	270	0	0	...	...	...	...	...	....	20	380	95	...	...	...
10	April 13...	300	0	0	...	...	...	...	...	....	2	134	98	...	...	...
	April 16...	300	0	0	...	...	...	...	...	....	2	500	99	...	...	...
	Total.....	5,967	3	.005	178	22	11	7	393	98+	742	4,229	85+	0	700	100

females carry the large additional posterior lateral spines but their other two posterior spines as well as their anterior spines were considerably larger in size than those of the control females.

Cultures containing ten drops of sodium silicate were very difficult to produce and consequently the majority of the experiments were carried on with jars containing five drops. Table I. shows the general results of the experiments and Figs. 1 and 2 show the dorsal views of the females from the control jars contrasted with those from the jars containing sodium silicate. The outline of the skeleton of each female was drawn with the camera lucida with the same magnification.

Table II.

Showing that the Females which Possess the Two Additional Posterior Lateral Spines in the Culture Medium Containing Sodium Silicate when Transferred to Culture Medium Free from Sodium Silicate Do Not Transmit the Spines to Their Descendants.

Experiments.	Observations, 1916.	Females with Spines transferred from a Jar Containing 5 Drops of Sodium Silicate in 150 c.c. of Culture Medium to a Jar of Culture Medium Free from Sodium Silicate on Feb. 22.		
		Offspring.		
		Females — Spines.	Females + Spines.	Females + Spines %.
1	Feb. 28.....	99	1	1
2	Feb. 28.....	95	5	5
3	Feb. 28.....	300	0	0

When the females of the *Brachionus amphiceros* type possessing the posterior lateral spines were transferred to jars of culture medium free from sodium silicate the descendants of these females did not develop the lateral spines but developed into the *Brachionus pala* type. Table II shows the results of such experiments.

Whether the rotifers were able to take the silicate out of the water in solution or whether the green flagellates first took it up and then the rotifers eating them obtained the silicate for their additional spines is not certain. A few experiments were performed in an attempt to determine this point. Green *Chlamydomonas* were reared in 150 c.c. culture medium containing 5–7 drops of sodium silicate. When the flagellates had become very numerous and supposedly had taken up as much of the sodium silicate as was possible they were removed from the siliceous medium and put into fresh medium free from sodium silicate. Several dozen rotifers lacking the posterior lateral spines were added. Eight days later two thousand females were

observed and only one possessed the posterior lateral spines. If the *Chlamydomonas* contained sodium silicate the rotifers in using them for food ought to have obtained enough of it with which to have formed the additional spines. When, however, the *Chlamydomonas* were transferred from the siliceous medium to the medium free from sodium silicate the siliceous materials which their bodies may have contained may have diffused out into the general medium, thus leaving their bodies practically free from the siliceous materials and consequently having no effect upon the rotifers.

It is, of course, quite possible that the siliceous material is not used at all in the formation of new spines and the increase in size of the other spines but that the sodium silicate, in some unknown way, stimulates each organism so that it takes out of the water more skeletogenous building material of another kind than under normal conditions. As yet, it is not known that the skeleton contains any siliceous material but it is considered to be entirely of chitinous material. If this is true the effect of sodium silicate upon the rotifers in causing an increase in the number and size of their spines is certainly not clear.

The fertilized eggs that were produced by the spined females of the *Brachionus amphiceros* type in the cultures containing sodium silicate produced females of the *Brachionus pala* type entirely lacking the lateral posterior spines of their mothers. 278 fertilized eggs from such mothers were allowed to develop and none of the females that they produced possessed the additional spines. Sachse and Powers found also that the fertilized eggs of the polymorphic females which they observed always developed into females of one particular type.

Summary.

1. Socium silicate added to the culture medium in which the rotifer *Brachionus pala* was living caused nearly all and in many cases caused all of the descendants to form two additional posterior lateral spines and also to increase the size of the other spines thus forming *Brachionus amphiceros*.

2. The parthenogenetic eggs from females reared in sodium silicate solutions developed into young females of the *Brachionus*

amphiceros type possessing these two lateral spines while the fertilized eggs from females reared in sodium silicate solutions developed into young females of the *Brachionus pala* type lacking these two lateral spines.

3. The variety or species of rotifer, *Brachionus amphiceros*, does not exist as a true variety or species, but is only one of the polymorphic stages of *Brachionus pala*.

4. Perhaps the polymorphic forms of this rotifer as it occurs in pools and ponds during the year are produced by the varying amounts of soluble siliceous substances in the water.

5. The females of *Brachionus amphiceros* possessing the two lateral posterior spines readily produced female parthenogenetic eggs, male parthenogenetic eggs, or fertilized eggs according to the supply of the green food *Chlamydomonas*.

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