FACTORS AFFECTING MALE-PRODUCTION IN HYDATINA[1]

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ONE FIGURE

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INTRODUCTION

After it had been well established that certain agents reduce the number of male-producing females in the families of the rotifer Hydatina senta, two important related lines of investigation were undertaken. One sought to explain how these agents operated to prevent the appearance of male-producers; the other had for its object the discovery of means of increasing the number of male-producers. The former line of research has proven nearly fruitless; the latter has met with some success. The experiments described in this paper were aimed at the solution of these two sets of problems.

[1] Contributions from the Zoölogical Laboratory of the University of Michigan.

EXPERIMENTS ON THE MECHANISM OF THE PREVENTION OF MALE-PRODUCTION

Osmotic pressure

The list of substances which effect a reduction in the number of male-producing females includes ammonium salts, sodium hydroxide, beef extract, manure solution, creatin, urea, and some others. In substances of such widely different properties it is difficult to select a common feature to which their common effect upon the life cycle of the rotifers could be attributed. One possibility that early suggested itself was that the osmotic pressure of the solutions produced the effect observed. Were this the only cause of the reduction in the number of male-producers, it would be expected that those solutions whose osmotic pressure was the highest would reduce male-production the most. Unfortunately it is probably quite impossible to say whether a solution produces osmotic effects in living protoplasm unless that protoplasm changes volume. Tables of osmotic pressures are therefore of little value in determining whether, in the experiments referred to, the reduction in the number of male-producers was proportional to the osmotic effect of the agent employed. As methods of detecting change of volume of the living rotifers or their tissues were found impracticable, it was necessary to resort to conjecture. In the experiment first to be described, a substance was selected which, if the tissues of the rotifers behaved as theoretically perfect semipermeable membranes, would give an osmotic pressure considerably higher than that which obtained in the other solutions used. The substance selected was cane sugar.

Experiment 1. Two sisters isolated January 13, 1911, became the parents of the two lines of this experiment, one of which was reared continuously in sugar solution, the other in distilled water. Food and other conditions were the same for both lines. A $\frac{M}{3}$ solution of cane sugar was kept in stock. It was heated daily to prevent fermentation, and was tested at intervals for inversion to reducing sugar. Once when Fehling's solution was reduced, the stock solution of sugar was rejected and a new one prepared. This stock solution was diluted to $\frac{M}{33}$ for use in the experiments.

The effect of the sugar solution, as shown in table 1, was to reduce the number of male-producers. But the amount of reduction was less than was expected on the theory that osmotic pressure was responsible for that reduction. It was less than the effects of certain ammonium salts whose effects should have been, theoretically, less than those of the sugar.

TABLE 1

Two lines of the rotifer Hydatina senta derived from sisters, one line reared in distilled water, the other in a $\frac{M}{33}$ solution of cane sugar. The number of male-producing (♂ ♀) and female-producing (♀ ♀) females is recorded for each line. The cane sugar reduces the number of male-producers

DISTILLED WATER				$\frac{M}{33}$ CANE SUGAR SOLUTION			
Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀	Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀
	Jan.				*Jan.*		
1	15	3	20	1	15	2	16
2	17	4	16	2	17	1	22
3	19	3	17		17	0	9
4	21	4	21	3	19	3	33
5	22	5	29		19	1	25
6	24	2	26	4	21	4	20
7	26	7	37		21	6	12
8	27	26	5	5	23	7	27
9	29	6	18		23	2	10
10	31	17	34	6	24	0	13
	Feb.				25	1	21
11	2	23	31	7	26	3	15
					27	9	14
				8	29	2	29
					29	5	23
				9	31	4	14
					31	7	19
					Feb.		
				10	2	9	14
					2	1	10
Total		100	254			67	346
Percentage of ♂ ♀ ..		28.2				16.2	

Acidity

In one of Shull's ('11) earlier experiments it was found that sodium hydroxide reduced the proportion of male-producers to a slight extent. At the same time experiments with acids were performed in the hope of obtaining the opposite effect, but it was found impossible to rear the rotifers in even a very dilute solution of the inorganic acids used. Although weak solutions of hydrochloric acid were used, by the time the slightly alkaline food was added, the solution appeared neutral. When he used solutions of the acid strong enough to remain acid after the food was added, the rotifers died.

With the expectation that organic acids might be less deleterious, the following experiment with butyric acid was begun.

Experiment 2. A 1 per cent stock so'ution of butyric acid was kept in a glass stoppered bottle, and diluted for use to 0.03 per cent (the diluent being Great Bear spring water). Two lines of rotifers derived from two sisters isolated September 10, 1911, were reared, one in spring water, the other in butyric acid solution. For want of a satisfactory indicator, it was not known that the latter solution remained acid after the food was added. The characteristic odor of butyric acid remained, but it was to be expected that the butyrates would possess the same odor.

Contrary to our hopes, the acid reduced[2] the proportion of male-producers, as shown in table 2.

Delay or inhibition by manure solution

The non-occurrence of male-producers while the rotifers were being reared in strong manure solution might be attributed to delay, rather than to inhibition. So long as a line were reared continuously in manure solution the delay would be continuous; but if only one or two generations were reared in such a solution, might not male-production which could not occur in these

[2] It may be pointed out that the families are larger in butyric acid (mean size, 47.1 daughters) than in spring water (mean size, 38.7 daughters). According to Mitchell's conclusions ('13) based on Asplanchna but applied to rotifiers in general, the higher nutrition evidenced by larger families should have been accompanied by greater male-production, instead of less.

TABLE 2

These two lines of Hydatina senta, derived from sisters, one line reared in spring water, the other in dilute butyric acid, show that butyric acid reduces the proportion of male-producers. It also increases the size of family

SPRING WATER				0.03 PER CENT BUTYRIC ACID			
Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀	Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀
	Sept.				*Sept.*		
1	12	0	46	1	12	3	51
2	14	4	19	2	15	0	42
3	16	6	23	3	17	0	52
4	17	0	26	4	18	0	45
5	19	0	28	5	20	2	46
6	20	7	45	6	22	1	45
7	22	11	44	7	23	4	39
8	23	7	44				
Total		35	275			10	320
Percentage of ♂ ♀		11.2				3.0	
Average size of family		38.7				47.1	

generations occur in greater degree in the generations immediately following them, which were reared in spring water? The possibility was tested in the following experiment.

Experiment 3. The general plan of the experiment was to rear, in spring water, females whose grandmothers had been kept in manure solution, and whose mothers were reared (except for a few hours after hatching) in spring water. The details of the method were as follows: Six or eight daughters of one female were divided into two lots of three or four each, one being put into spring water, the other into manure solution. Eight to 24 hours later these lots were transferred to new dishes, one in spring water as before, the other in manure solution. The transfer was made in order that the first young to hatch in the second dish of manure solution might be known to have hatched from eggs that underwent their maturation and part of their previous development in manure solution. These first young, if females, were transferred to spring water, in which (if they proved to be female-producers) they were kept the rest of their lives. Their entire families were reared to maturity and recorded. The totals for each lot of families simultaneously reared are shown in table 3.

TABLE 3

Records of male-producers and female-producers among females whose grandmothers were reared in manure solution, and females whose grandmothers were reared in spring water. All individuals other than these grandmothers, of all generations, and in both halves of the experiment, were reared in spring water. There is no accumulation of male-producers among granddaughters of females reared in manure solution

LOT NUMBER	GRANDDAUGHTERS OF FEMALES REARED IN SPRING WATER		LOT NUMBER	GRANDDAUGHTERS OF FEMALES REARED IN MANURE SOLUTION	
	Number of ♂ ♀	Number of ♀ ♀		Number of ♂ ♀	Number of ♀ ♀
A	9	44	A	32	37
B	4	51	B	1	38
C	2	40	C	4	70
D	33	44	D	30	37
E	9	13	E	19	19
F	28	16	F	15	83
Total.......	85	208		101	284
Percentage of ♂ ♀...	29.0			26.2	

There is no increase in the proportion of male-producers among the granddaughters of females bred in manure solution. The figures show an actual decrease, but it is so small as to be probably insignificant. The manure solution does not merely delay, but inhibits, male-production.

EXPERIMENTS DESIGNED TO INCREASE MALE-PRODUCTION

In an attempt to increase male-production artificially a number of salts were tested, some of them being selected because of their well-known physiological effects in no way connected with sex, others selected purely at random. Only those in which the rotifers could be easily reared are reported in this paper. While this work was in progress Whitney's ('14) paper on nutrition experiments appeared. Our energy was then directed toward testing the agents which seemed to us to enter into Whitney's experiments but which he had apparently neglected. These two lines of work are discussed separately.

EFFECT OF VARIOUS SALTS

Experiment 4. Calcium chloride.[3] Two sister individuals of a line obtained in Nebraska were isolated on November 20 and 21, respectively, 1912. One, with its progeny, was reared in spring water, the other, with its progeny in $\frac{N}{100}$ solution of calcium chloride. Other conditions were the same in both lines. As shown in table 4, the only male-producers in this experiment appeared in the calcium chloride solution.

TABLE 4

The two lines of rotifers in this table were reared, one in spring water, the other in a dilute solution of calcium chloride. The only male-producers (♂ ♀) in all the families were produced in calcium chloride

SPRING WATER				$\frac{N}{100}$ CALCIUM CHLORIDE			
Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀	Number of generation	Date of first young	Number of ♂ ♀	Number of ♀ ♀
	Nov.				*Nov.*		
1	22	0	25	1	24	0	31
2	24	0	39	2	26	0	42
3	26	0	26	3	28	0	45
4	29	0	24	4	30	2	40
	Dec.				*Dec.*		
5	2	0	26	5	2	0	23
6	4	0	42	6	4	0	47
7	5	0	44	7	8	0	23
8	7	0	24	8	10	0	12
9	9	0	22	9	13	0	45
10	12	0	1	10	15	0	21
	14	0	9				
11	15	0	50				
Total..............		0	332			2	329
Percentage of ♂ ♀ ..		0.0				0.6	

Experiment 5. More dilute calcium chloride. The above experiment was repeated, using a more dilute solution of calcium chloride. A $\frac{N}{300}$ solution was used in the first three generations, $\frac{N}{600}$ thereafter. In table 5, which records the details, it appears that three times as many male-producers were reared in calcium chloride as in spring water.

Experiment 6. Calcium chloride used intermittently. In this experiment, only one line was reared, beginning Jan. 11, 1913. Instead

[3] The results of these experiments with calcium chloride have been published in summary form elsewhere (Shull, '13) but appear here for the first time in detail.

TABLE 5

One of the two lines here recorded was reared in spring water, the other in a solution of calcium chloride more dilute than that used in table 4. Again there were more male-producers in the calcium chloride

SPRING WATER				$\frac{N}{300}$ TO $\frac{N}{600}$ CALCIUM CHLORIDE			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	Jan.				*Jan.*		
1	13	2	40	1	13	0	38
2	15	0	40	2	15	0	15
3	17	0	24	3	17	0	1
4	18	0	35	4	18	0	29
5	20	0	49	5	20	9	31
6	22	5	35	6	22	7	43
7	24	0	33	7	24	0	40
8	26	0	29	8	26	4	44
9	28	0*	7*	9	28	0*	11*
Totals		7	292			20	252
Percentage of ♂♀		2.3				7.3	

* Remainder of family not recorded.

of rearing it continuously in one medium, alternate generations were reared in spring water and a $\frac{N}{600}$ solution of calcium chloride. All members of one family were reared, from the time they were isolated shortly after hatching, in the same medium. That member of each family which was selected to become the parent of the next generation laid her eggs in the medium in which she was bred, and her eggs hatched there. But shortly after hatching, her daughters were transferred to the alternate medium, and there reached maturity.

Most of the male-producers appearing in this experiment, as shown in table 6, were offspring of females that lived and laid their eggs in calcium chloride solution. Since it is the growth and maturation period of an egg in which it is determined whether a male-producer or a female-producer will develop from that egg, as shown by the earlier experiments of Shull ('12), the results given in table 6 are what would be expected if calcium chloride favors male-production. In this respect Experiment 6 is in accord with the other calcium chloride experiments above.

TABLE 6

In the single line here recorded the first generation was reared in spring water (from a parent reared in $\frac{N}{500}$ calcium chloride); the second generation was reared in a calcium chloride solution (from a parent reared of course in spring water). Most of the male-producers are daughters of parents reared in calcium chloride

NUMBER OF GENERATION	DATE OF FIRST YOUNG	OFFSPRING OF PARENTS REARED IN SPRING WATER		OFFSPRING OF PARENTS REARED IN CALCIUM CHLORIDE	
		Number of ♂♀	Number of ♀♀	Number of ♂♀	Number of ♀♀
	Jan.				
1	13			0	26
2	15	0	14		
3	17			0	25
4	19	1	15		
5	21			4	41
6	23	0	23		
7	25			6	32
8	27	0	27		
Total		1	79	10	124
Percentage of ♂♀		1.2		7.4	

Experiment 7. Calcium chloride and a New Jersey line of rotifers. The effect of continuous rearing in calcium chloride of two concentrations was tested in a line of rotifers from New Jersey, to check the results in the three preceding experiments, in all of which the Nebrasla line was used. The method of conducting the experiment was the same, except that different concentrations were used, but, as may be seen from table 7, the results were different. There is a reduction in the number of male-producers in calcium chloride in this line.

We are unable to explain the difference between the Nebraska and the New Jersey line in this respect, though inherited physiological differences of other kinds are well known. The constancy of the results obtained with calcium chloride in the Nebraska line, even if the differences produced were small, lead us to attribute these effects with some confidence to the action of the calcium chloride.

Experiment 8. Magnesium chloride. The physiological effect of magnesium upon other organisms is in some ways in strong contrast to the effect of calcium. We hoped to find this difference extended to their effect upon the life cycle of Hydatina. A $\frac{N}{150}$ solution of magnesium chloride was used for one line, spring water in another

TABLE 7

Three lines of rotifers of a stock obtained in New Jersey were started from two sisters. One line was reared in spring water, the other two in two concentrations of calcium chloride. There are fewer male-producers in the calcium chloride solutions

SPRING WATER			$\frac{N}{200}$ CALCIUM CHLORIDE			$\frac{N}{75}$ CALCIUM CHLORIDE		
Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀
1	0	4	1	0	23	1	1	30
2	3	38	2	6	31	2	0	31
3	17	27	3	0	38	3	0	34
4	0	27	4	4	14	4	2	31
5	0	27	5	0	15	5	0*	15*
6	0*	11*	6	0*	10*	6	0*	13*
7	0*	14*	7	0*	11*	7	0*	8*
8	0	42	8	0	36	8	0	26
9	0	29	9	0	3	9	0	2
10	0	2		0	4			
	0	3	10	0	9			
Total..........	20	224		10	194		3	190
Percentage of ♂♀..........	8.1			4.8			1.5	

*Remainder of family not recorded.

line. Although both lines were reared for ten or more generations and over three hundred individuals were obtained in each line, not a single male-producer appeared in either line. It seems unnecessary to state such uniform results in detail, hence no table for this experiment is published.

There is no way of interpreting the results of this experiment. Magnesium chloride may reduce male-production. Or it may increase male-production, but could not overcome a strong 'tendency' to female-production in the line used. The experiment has never been repeated with a line in which male-producers appeared more frequently.

Experiments 9, 10, and 11. Potassium sulfate, iron chloride, and ammonium chloride. The success obtained by using very dilute calcium chloride suggested that very dilute solutions of other substances might also increase male-production, even in the case of substances, more concentrated solutions of which were known to have the reverse effect. Among the substances tested were the three named above. Since all of them reduced male-production or showed no effect, the results of the several experiments are for brevity summarized in a single table (table 8).

TABLE 8

Summaries of several experiments with dilute solutions of potassium sulphate, iron chloride, and ammonium chloride. The number of male-producers is not increased by any of these substances, but in most cases is reduced

EXPERIMENT						CONTROL			
Substance	Concentration	Number of generations	Number of ♂♀	Number of ♀♀	Per cent of ♂♀	Number of generations	Number of ♂♀	Number of ♀♀	Per cent of ♂♀
Potassium sulphate	$\frac{N}{200}$	8	19	343	5.2	8	31	326	8.6
Potassium sulphate	$\frac{N}{50}$	8	28	269	9.4	8	31	326	8.6
Iron chloride.......	$\frac{1}{600}\%$	5	8	158	4.8	5	43	157	21.5
Ammonium chloride..........	$\frac{1}{1000}\%$	9	0	241	0.0	10	3	356	0.8

FACTORS SUGGESTED BY WHITNEY'S EXPERIMENTS

Whitney ('14) reared Hydatina on a diet of Chlamydomonas, and obtained a striking increase in the proportion of male-producers. He freely attributed this effect to the (qualitative) difference in nutrition, without mentioning several agents whose possible effects had not been eliminated. For example, his food cultures were started in different media; Chlamydomonas was reared in a solution of bouillon cubes, the Polytoma used in the control was reared in manure solution. Although only small quantities of the liquids of the food cultures were introduced with the food, nevertheless there must have been some initial differences of a chemical nature between the experiment and its control. The possible effects of these substances were not tested, nor were they mentioned in Whitney's paper. Furthermore, it was probable that the organisms in the food cultures produced, as a result of their metabolic activities, differences between the food cultures that did not exist at first. Thus, it might be expected that Chlamydomonas, a green organism, would carry on photosynthesis, with the resultant liberation of oxygen. It seemed not improbable that oxygen might produce part of the effects in the experiments with Chlamydomonas. This was the more plausible since, as Whitney states, marked effects were produced only when large numbers of Chlamydomonas were present, and only when the dishes were kept in direct sunlight. Both of the conditions named should result in the production of relatively large quantities of oxygen in solution.

If it should appear that any or all of these agents present in the Chalmydomonas cultures have any considerable effect in increasing male-production, Whitney's conclusion that his experiments gave evidence of an effect of nutrition would lose much of its support. If, on the other hand, these obvious factors could be shown to have no effect whatever, the belief that nutrition effected the increase of male-production noted would be greatly strengthened.

It may be stated in advance that our results may be interpreted as being largely in support of Whitney's contention; for, while one of the suspected agents (oxygen) gave positive results, its effect was much smaller than that which Whitney reported. Our experiments are the more significant because we have worked with a line of rotifers kindly sent to us by Dr. Whitney in January, 1915, from the line used in his own experiments. We can only regret that our experiments were not conducted by Dr. Whitney himself, as we have had some difficulty in duplicating his conditions merely from his published accounts.

In the account which follows, we separate the experiments aimed to test the effect of dilute bouillon from those designed to test the effect of oxygen.

Effect of dilute bouillon

Experiment 12. The bouillon solution used in this experiment was made from one cube of Armour's beef bouillon in 21,000 cc. of water. As nearly as we could compute from Whitney's paper, this was the concentration of bouillon present in his cultures after the rotifers had been fed with Chlamydomonas. Two lines of rotifers, derived from sisters, were reared, one in spring water, the other in this dilute bouillon solution. Table 9 shows a slight reduction in the number of male-producers in bouillon, rather than an increase.

TABLE 9

Showing the effects of continuous rearing of Hydatina in a dilute bouillon solution. The number of male-producers (♂♀) is slightly decreased

SPRING WATER			BOUILLON		
Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀
1	0	15	1	0	11
2	6	17	2	3	32
3	2	19	3	0	3
4	0	17	4	0	17
5	0	29	5	0	8
6	1	24	6	1	29
7	0	14	7	0	20
			8	1	25
Total.......	9	135		5	145
Percentage of ♂♀...	6.2			3.3	

Experiment 13. Bouillon was tested in this experiment by rearing only the parents in bouillon, not their offspring. This was Whitney's method in working with Chlamydomonas, and was based on Maupas's statement (1891), subsequently confirmed by Shull ('12), that the determination of the sex of an individual takes place in the body of its grandmother. The concentration of the bouillon was made less than that in Experiment 12, being one cube in quantities of water varying, on different days, from 30,000 to 80,000 cc. Two lots of females of approximately equal size and from the same source were set aside on the same day, the one in spring water, the other in bouillon solution. The parents were removed after 24 hours, The offspring from all eggs laid in that time were reared to maturity in spring water, and recorded. In table 10 the nature of these offspring is summarized. Here again there is a slight reduction in the number of male-producers in bouillon solution. The consistent results of these two experiments seemed to make it not worth while to look for a male-producing factor in the dilute bouillon solution used by Whitney.

TABLE 10

Showing the effects, on the offspring, of rearing the parents in dilute bouillon. The eggs from which the offspring hatched were laid and hatched in bouillon in one-half of the experiment, those of the other half laid and hatched in spring water. In both cases the offspring were removed to spring water a few hours after hatching

DATE	PARENTS REARED IN SPRING WATER			PARENTS REARED IN BOUILLON		
	Number of parents	Daughters proved to be		Number of parents	Daughters proved to be	
		♂♀	♀♀		♂♀	♀♀
January 27	3	2	10	4	0	11
30	4	0	59	4	2	42
February 5	3	0	39	4	0	65
9	3	0	28	3	0	27
16	3	18	20	3	9	38
22	3	0	54	3	0	52
March 1	2	3	7	3	0	8
Total	21	23	217	24	11	243
Percentage of ♂♀		9.5			4.3	

Effect of metabolic products of a green alga

In the hope of approximating the conditions of Whitney's experiments except the nutritive conditions, we reared the rotifers in water containing a green alga that the animals could not eat. Spirogyra was selected, though it was not known whether the metabolic products of Spirogyra are similar to those of Chlamydomonas or not.

Experiment 14. In this experiment, one of two lines derived from sisters was reared in water in which Spirogyra was kept, the other in water without Spirogyra. The same food was used for both. The Spirogyra was obtained from a spring, was kept in dishes in direct sunlight in the laboratory, and was washed out several times in Great Bear spring water before using, to prevent the introduction of foreign water in one part of the experiment. In the Spirogyra line only the parents and the first three or four daughters were kept in dishes with Spirogyra. The young females were removed daily to Great Bear water, in which they were reared to maturity. All dishes containing the parents, whether with Spirogyra or not, were in direct sunlight a part of the day. The temperature was not high when these experiments were performed (late winter and early spring), hence it was not necessary to take any precautions to reduce the temperature of dishes

TABLE 11

Showing the effect of rearing rotifers in the presence of Spirogyra. In one line the parents of each generation were reared in dishes containing Spirogyra; in the other line there was no Spirogyra in the parents' dishes. All offspring in both lines were reared in the absence of the alga

PARENTS REARED WITHOUT SPIROGYRA			PARENTS REARED WITH SPIROGYRA		
Number of generation	Number of ♀ ♀	Number of ♀ ♀	Number of generation	Number of ♂ ♀	Number of ♀ ♀
1	8	42	1	14	41
2	11	42	2	12	28
3	1	38	3	2	18
4	2	18	4	7	43
5	3	22	5	6	36
Total.........	25	162		41	166
Percentage of ♂ ♀.....	13.3			19.8	

set in the sunlight. Bubbles, presumably of oxygen, were usually present in the Spirogyra dishes, indicating that photosynthesis was taking place.

As indicated in table 11, there is a noticeable increase in the number of male-producers in the Spirogyra cultures.

Experiment 15. The preceding experiment was repeated, this time with two lines in Spirogyra cultures, and but one control. The methods were the same as described for Experiment 14. Table 12 gives the results. In these lines there is practically no difference between the Spirogyra cultures and the control.

Experiment 16. Adopting for this experiment Whitney's method of rearing only the parents under experimental conditions, we used the following procedure. Two or three mature, egg-laying females, members of the same family, were placed in each of two dishes. In one was placed some Spirogyra, in the other none. Both dishes were put into a covered dish, which was floated on the water in a small aquarium, and kept in the sunlight at a south window during the major part of each day. A thermometer was put into the floating dish, and the temperature (at this time often quite high out of doors) was found to vary only two or three degrees during the day. After 24 hours the parents were removed. The eggs laid during this 24 hour period hatched where they were laid, and the young were removed to spring water to grow to maturity. These offspring are recorded in table 13. In this experiment there is a slight reduction in the number of male-producers, though it is so small as to be probably insignificant.

TABLE 12

Showing the effects of continuous rearing of the rotifers in water containing Spirogyra, as in Table 11. Two lines were reared in Spirogyra cultures, with a single control

WITHOUT SPIROGYRA			WITH SPIROGYRA			WITH SPIROGYRA		
Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀	Number of generation	Number of ♂♀	Number of ♀♀
1	1	10	1	0	8	1	0	32
2	1	30	2	1	19	2	8	16
3	2	10	3	0	33	3	0	16
4	3	32	4	0	19	4	1	23
5	1	9	5	3	21	5	4	51
6	3	42	6	2	20	6	0	19
7	0	13	7	0	13	7	0	3
			8	0	10			
			9	6	37			
Total...........	11	146		12	180		13	160
Percentage of ♂♀..........	7.0			6.2			7.5	

TABLE 13

Recording the offspring of parents reared in Spirogyra cultures, as contrasted with those of parents reared in the absence of Spirogyra. All offspring were reared in spring water. Spirogyra used in this way does not increase male-production. See also tables 11 and 12

WITHOUT SPIROGYRA			WITH SPIROGYRA		
Number of parents	Daughters		Number of parents	Daughters	
	Number of ♂♀	Number of ♀♀		Number of ♂♀	Number of ♀♀
3	3	15	2	0	14
3	5	18	3	2	22
2	2	40	2	7	35
2	7	28	2	4	22
2	1	19	2	1	18
Total.........	18	120		14	111
Percentage of ♂♀........	13.0			11.2	

On the basis of the Spirogyra experiments as a whole, we are unable to draw a definite conclusion. While by continuous rearing with Spirogyra an increase in the number of male-producers was one time obtained, at another time the increase was practically zero; while when only the parents were reared in Spirogyra cultures, the offspring were slightly less frequently male-producers than in the control.

Effect of oxygen

The effect of oxygen was directly tested in several experiments. The method, in general, was to put the rotifers into a dish of water previously saturated with a mixture of air and oxygen containing a known proportion of oxygen, then set the dish in an atmosphere containing the same proportion of oxygen. The two atmospheres used were composed, respectively, of 40 per cent and 60 per cent oxygen. The oxygen was obtained by heating potassium chlorate with manganese dioxide and collecting the gas in a gas holder over water. No attempt was made to purify the oxygen.

The 40 per cent oxygen atmosphere for saturating the water was obtained by lowering a graduated tube, fitted at one end with a stop-cock and open at the other end, into a vessel of water, admitting first three volumes of air, and then one volume of oxygen from the gas holder. The mixture was subsequently driven into a flask of spring water inverted over water, and later shaken repeatedly with the water remaining in the flask. When the rotifers were put into a dish of this oxygenated water, the dish was set under a bell jar made air-tight at the bottom with a vaseline-coated rubber gasket. By means of a filter pump, one-fourth of the air under the bell jar was removed, as indicated by a mercury manometer in connection with the bell jar. Oxygen from the gas holder was then admitted until the mercury fell to zero. The partial exhaustion and refilling of the bell jar occupied usually less than two minutes. The 60 per cent oxygen atmosphere was obtained in the same way, except that equal parts of air and oxygen were mixed.

Experiment 17. Continuous rearing in 40 per cent oxygen. Two sisters were isolated March 31, 1915, one in spring water, the other in similar water first saturated with an atmosphere composed of 40 per cent of oxygen. The former dish was kept in air, the latter under a bell jar in an atmosphere containing 40 per cent of oxygen. From each parent was reared a line under the same conditions as those in which the parent female was placed. In the oxygen line, only the parents and several additional members of each family were kept in oxygenated water. Four or five of the first daughters of each family were kept in oxygenated water, and from among them the parent of the next generation was selected. The remaining daughters in each family were transferred to spring water, where they reached maturity. Whatever effect the oxygen has, therefore, must be exerted in early larval stages, or in the egg, or in the body of the mother. From the previous experiments of Shull ('12) it is to be expected that the egg or oögonial stages are the only ones in which the life cycle can be altered.

TABLE 14

Recording two lines derived from sisters, one reared in untreated spring water, the other in water which was first saturated with an atmosphere of which 40 per cent was oxygen, then placed in a corresponding atmosphere. The oxygen line yields more male-producers than the control

AIR				40 PER CENT OXYGEN			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	Apr.				*Apr.*		
1	3	0	16	1	3	2	28
2	6	1	36	2	4	1	27
	6	0	29	3	6	2	32
3	8	0	8	4	8	4	46
	9	0	6	5	10	7	43
4	10	3	48	6	12	4	42
5	12	11	43	7	14	10	39
6	14	8	33	8	16	2	39
7	16	1	22	9	18	0	8
8	18	0	6		19	0	12
	19	0	8	10	20	0	14
9	20	0	23	11	22	12	38
10	22	4	23		22	4	35
11	23	1	35	12	23	2	44
12	25	1	35	13	25	9	25
Total		30	371			59	482
Percentage of ♂♀		7.4				10.9	

Table 14 records the results of this experiment. There is a small increase in male-production in the oxygen line.

Another feature of the oxygen line that is perhaps of significance in connection with the next experiment, is the distribution of the male-producers in the family. In any line of Hydatina the male-producers tend to occur in groups; that is, several successive members of a family will be male-producers. This grouping is most apparent in lines in which male-producers are abundant; for, though the most frequent number of male-producers in a group is always one, by far the majority of male-producers in such a line occur in much larger groups. When the proportion of male-producers is 30 to 40 per cent, it is not uncommon to find groups of ten, twenty, or even thirty or more male-producers occupying successive places in the family. The cause of this grouping is unknown, but evidently when the conditions are right for the appearance of male-producers, they remain so for a time, then disappear or are inoperative.

Were there no such grouping, in a line yielding only 5 or 10 per cent of male-producers, most of these would necessarily occur in groups of one. Groups of two would be very uncommon, while larger groups would be almost unknown.

It is interesting, therefore, especially in view of the fact that large groups are quite common in lines in which male-producers are abundant, to compare the distribution of the male-producers in the two lines of table 14, one having a higher proportion of male-producers than the other. The daily records of these two lines have been examined, and the number of times that one, two, three, four, or five successive members of the family were male-producers was recorded. There were no groups larger than five. If such records are to be trustworthy, it is essential that the order of individuals in the family be known. There is a possibility of error here, for the order of age was determined each day by the order of size. In the young females the differences in size usually left little doubt as to their relative ages, but errors were undoubtedly sometimes made. However, errors were no more probable in one line than in the other; and there could have been no bias, either conscious or unconscious, on our part, since the order was determined long before it was known which ones would be male-producers.

The number of groups of successive daughters, of one to five members, comprising only male-producers, is summarized in table 15. The slight increase in the mean number of male-producers per group in the oxygen line is not in proportion to

TABLE 15

Showing the number of times that groups of one, two, three, four, or five successive members of the families in table 14, were male-producers

NUMBER OF MALE-PRODUCERS OCCURRING IN SUCCESSION	NUMBER OF GROUPS IN AIR LINE	NUMBER OF GROUPS IN OXYGEN LINE
1	15	27
2	4	10
3	1	1
4	1	1
5	0	1
Mean size of group.......	1.42	1.47

the increase in the total number of male-producers in that line. The additional male-producers called forth by the oxygen appear, therefore, in additional groups of small size, rather than as additional members of the groups which would otherwise appear. Thus, there is an increase in the number of groups, rather than an increase in the size of the groups. This means a more uniform distribution of the male-producers through their respective families in the oxygen line.

Experiment 18. Continuous rearing in 60 per cent oxygen. This is a repetition of Experiment 17, but with an atmosphere of which 60 per cent was oxygen, instead of 40 per cent. The methods used were otherwise the same. Table 16 gives the results by generations. There is no increase in the number of male-producers in the oxygen line; whether the slight decrease is significant is not known.

Although there is practically the same proportion of male-producers in both lines in this experiment, the distribution of these male-producers over the families is more uniform in the oxygen line than in the control. This is shown first by the number of male-producers occupying successive places in the

TABLE 16

Showing number of male-producers (♂♀) and female-producers (♀♀) in two lines of Hydatina, one reared in water in ordinary air, the other in water saturated with an atmosphere of which 60 per cent was oxygen

AIR				60 PER CENT OXYGEN			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	June				*June*		
1	1	1	20	1	1	4	31
2	3	0	3	2	3	0	9
	4	0	1	3	5	2	22
	6	3	13		5	4	25
3	7	12	10	4	7	3	37
4	9	10	31	5	9	4	17
5	11	0	10	6	11	1	3
6	14	0	5	7	14	5	28
	14	0	1	8	17	16	16
	14	2	28	9	19	3	42
7	15	13	23	10	20	1	8
8	17	23	20		21	11	38
9	19	3	26	11	22	19	31
10	21	15	23	12	25	7	31
11	23	1	46	13	27	11	23
12	25	15	36	14	28	14	25
13	27	18	34	15	30	2	25
14	29	4	45		*July*		
15	30	6	32	16	2	6	11
	July			17	4	1	30
16	3	24	24	18	6	35	12
17	4	9	39				
18	6	24	29				
Total............		183	499			149	464
Percentage of ♂♀		26.8				24.3	

families. Table 17 records all these groups of successive male-producers in both lines. The average size of these groups in the air line is 3.26, in the oxygen line only 2.40. The male-producers are distributed more uniformly through the families in the oxygen line than in the control. In this connection, see also table 15, and the discussion of it under Experiment 17.

This difference in the distribution of the male-producers is also seen if, instead of recording the generations separately, as

TABLE 17

Showing the number of groups (of various sizes) of successive male-producers in the families of the two lines recorded in table 16

NUMBER OF MALE-PRODUCERS OCCURRING IN SUCCESSION	NUMBER OF GROUPS IN AIR LINE	NUMBER OF GROUPS IN OXYGEN LINE
1	32	33
2	4	18
3	4	2
4	3	3
5	5	2
6	2	1
7	0	2
9	3	0
12	1	0
19	1	0
24	1	0
32	0	1
Mean size of group of successive male-producers..................	3.26	2.40

in table 16, one examines the daily output. In each day's product occur members of several successive generations. As Shull (1915) has pointed out, rhythmical production of male-producers may often be more easily detected by an examination of daily records than by a study of family records. In table 18 is recorded the number of male-producers and female-producers on each day in each line of table 16, regardless of the families to which they belong. The percentages of male-producers are computed for tri-daily periods, in order to smooth some of the enormous fluctuations in both lines. These tri-daily percentages are graphically shown in figure 1, where it is seen that the oxygen line is plainly more uniform than the control line.

If it be objected that the daily records should not be combined for comparison, that combination may be rejected, and the difference between the two lines is still plainly visible. The number of male-producers appearing on successive days in the control line is subject to greater extremes of fluctuation than in the oxygen line. Of the 39 days through which the experiment extended, there were twelve days on which no male-producers ap-

TABLE 18

Showing the number of male-producers and female-producers appearing each day in the families recorded in table 16, regardless of the families to which they belonged

DATE	AIR LINE			60 PER CENT OXYGEN LINE		
	Number of ♂♀	Number of ♀♀	Per cent of ♂♀	Number of ♂♀	Number of ♀♀	Per cent of ♂♀
June						
1	0	2		0	1	
2	0	5	5.8	1	11	10.7
3	1	9		2	13	
4	0	0		0	6	
5	0	8	15.7	1	11	12.5
6	3	8		4	18	
7	1	8		1	23	
8	4	4	50.0	1	16	10.7
9	11	4		5	19	
10	6	7		2	6	
11	0	13	15.0	0	10	14.2
12	0	14		1	2	
13	0	6		0	1	
14	0	6	0.0	0	7	0.0
15	0	6		0	6	
16	2	18		2	3	
17	0	17	22.2	5	10	37.9
18	10	7		4	5	
19	7	13		7	19	
20	10	12	44.2	5	16	17.6
21	10	9		0	21	
22	3	22		2	15	
23	6	13	26.2	7	22	27.1
24	7	10		10	14	
25	1	21		4	17	
26	1	23	10.2	5	13	28.5
27	6	26		9	15	
28	9	21		4	20	
29	17	13	29.5	9	15	22.0
30	3	35		4	25	
July						
1	2	29		6	21	
2	0	11	11.9	4	7	25.0
3	6	19		2	8	
4	5	11		2	7	
5	14	11	44.0	5	6	26.6
6	14	20		1	9	
7	10	16		9	18	
8	14	15	38.7	13	6	56.6
9	0	7		12	2	

peared in the control line, only seven such days in the oxygen line. On the other hand, there were nine days on which the control line yielded ten or more male-producers, only three such days in the oxygen line. The second of the above-mentioned differences may be partly due to the greater absolute number of male-producers in the control line; but if allowance be made for this fact, the former difference becomes all the more striking.

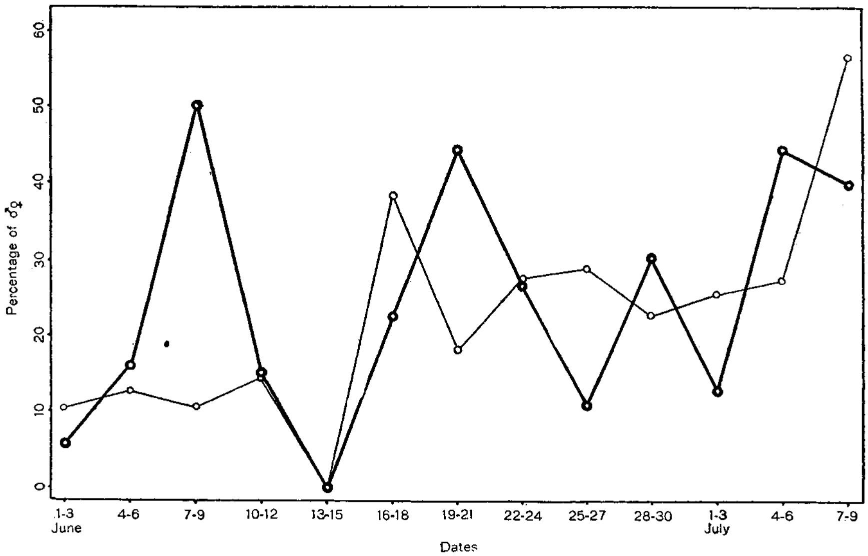

Fig. 1 Graphic representation of the proportion of male-producers, computed for three-day periods, in the two lines recorded in tables 16 and 18. The light curve represents the oxygen line, the heavy curve the control. The fluctuation is less in the oxygen line.

A further difference between the two lines is the existence of four fairly distinct waves of male-production in the control line, each separated from the others by periods of few male-producers. Such a rhythm in the oxygen line is less distinct, or in part wanting.

These differences are all due to a more uniform distribution of the male-producers over the families of the oxygen line, and over the period of the experiment in the oxygen line, than in the control. Thus, although the oxygen did not cause an increase in the number of male-producers, it was not without its effect upon male-production.

Why the 60 per cent oxygen in this experiment did not increase male-production, whereas the 40 per cent oxygen of the preceding experiment did, is not known. Different concentrations of the same agent may have different effects. Or oxygen may increase male-production only when the other conditions present are rather unfavorable to male-production. That the other conditions were right for high male-production is shown by the fact that the control line produced 26.8 per cent of male producers, as against 7.4 per cent in Experiment 17. The stock of rotifers used was, at the time of the experiment, in one of its waves of high male-production. This may be the reason why the oxygen could not still further increase the number of male-producers.

Experiment 19. Oxygen counteracting bouillon. This and the following three experiments were suggested by the possibility, mentioned in the preceding paragraph, that oxygen could increase male-production only when other conditions were rather unfavorable to male-production. The several experiments immediately following show the influence of oxygen in counteracting the effects of agents known to reduce the number of male-producers.

In this experiment, a one-seventh per cent solution of Armour's beef bouillon cubes was used. Only the parents were reared in the bouillon, not the offspring (this being Whitney's method). The parents were put into bouillon for 10 to 14 hours, then transferred to a new dish of bouillon. This transfer was made to insure that all the eggs laid in the second dish went through their maturation stages in the bouillon. The bouillon in the second dish was first saturated with an atmosphere containing 40 per cent oxygen, and the dish was then placed under a bell jar, in a 40 per cent oxygen atmosphere. The parents remained in the second dish for twenty-four hours, at the end of which time they were removed. All the eggs laid in the second dish of bouillon were allowed to hatch there, after which the young were transferred to spring water, where they grew to maturity.

The control parents were kept only in spring water which was kept in ordinary air. Every time the parents in bouillon were transferred to a new dish, the control parents were likewise transferred to fresh spring water, to prevent the unequal accumulation of metabolic products in the two lines.

Table 19 shows the results of the experiments. The oxygen appears not only to have counteracted the effect of the bouillon, but to have added a substantial balance of male-producers on the bouillon side.

TABLE 19

Showing the effect of oxygen and bouillon on one line, as contrasted with a line not subjected to either. Bouillon, even in dilute solutions, has been shown to reduce the number of male-producers (see table 9). In this experiment, oxygen counteracts the effect of the bouillon, and actually increases the proportion of male-producers above that in the control line

SPRING WATER, WITHOUT OXYGEN			BOUILLON, WITH OXYGEN		
Number of experiment	Number of ♂♀	Number of ♀♀	Number of experiment	Number of ♂♀	Number of ♀♀
A	3	23	A	1	24
B	1	11	B	10	4
C	1	23	C	0	8
D	0	14	D	0	3
E	0	16	E	0	23
F	0	26	F	0	12
G	0	13	G	0	9
H	1	22	H	6	12
I	0	3	I	0	11
J	0	22	J	14	20
K	0	6	K	0	2
Total............	6	179		31	128
Percentage of ♂♀	3.2			19.5	

Experiment 20. Oxygen counteracting strong manure solution. In this experiment only the parents were reared in the manure solution, the offspring being in all cases reared in spring water. The parents to be used for both experiment and control were placed in manure solution in the afternoon; the next morning one lot (the control) was transferred to a new dish of manure solution, the other lot was transferred to manure solution which had been saturated with an atmosphere of which 40 per cent was oxygen. The former lot was kept in air, the latter under a bell jar in an atmosphere of 40 per cent oxygen. At the end of 24 hours the parents were removed from both dishes. All eggs laid in the 24 hour period were allowed to hatch where laid, the young females being then removed to spring water.

The young females from each lot of parents were transferred to spring water on two successive days. When the parents were removed, those eggs that were laid in the first seven to ten hours had already hatched. These females were transferred to spring water at that time; in table 20 they are recorded as 'older daughters.' On the following day all the remaining eggs

TABLE 20

The parents were reared in strong manure solution, but in one-half of the experiment this solution was saturated with an atmosphere 40 per cent of which was oxygen. All offspring were reared in spring water. The oxygen approximately doubled the proportion of male-producers

DATE	MANURE SOLUTION, AIR					MANURE SOLUTION, 40 PER CENT OXYGEN				
	Number of parents	Older daughters		Younger daughters		Number of parents	Older daughters		Younger daughters	
		♂♀	♀♀	♂♀	♀♀		♂♀	♀♀	♂♀	♀♀
Apr.										
28	5	0	17	0	12	4	0	13	0	13
29	3	1	1	3	17	3	0	4	0	8
30	3	0	7	0	9	3	0	4	0	1
30	2	0	2	0	6	2	1	5	0	5
May										
4	2	0	0	0	15	2	0	4	1	12
4	2	0	3	0	13	2	0	4	2	11
5	2	0	5	0	17	2	0	3	0	6
5	2	0	5	0	24	2	0	9	0	17
6	2	0	5	1	13	3	0	18	0	15
6	4	0	12	1	37	3	0	17	0	19
7	2	0	6	0	14	3	0	11	0	18
7	4	0	9	0	29	3	0	9	0	16
9	4	0	19	2	48	4	0	17	3	33
10	3	1	8	0	26	4	0	25	1	38
12	3	0	22	0	23	2	2	16	1	18
14	3	0	6	0	25	3	0	8	1	16
16	3	0	11	3	17	2	0	7	1	11
18	1	0	2	1	8	3	0	4	2	5
20	4	2	8	0	7	4	2	13	0	22
22	3	0	14	0	7	2	0	7	3	10
24	4	3	16	2	22	4	6	23	6	23
26	3	0	1	0	17	3	1	5	0	21
28	5	0	13	0	50	5	3	21	6	33
30	3	0	2	0	20	3	2	4	0	10
Total..	72	7	194	13	476	71	17	251	27	381
Percentage of ♂♀		3.4		2.6			6.3		6.6	

had hatched; the young females were removed to spring water, and appear in table 20 as 'younger daughters.' The sex-ratio for the older and younger daughters is given separately to show that the effect of the oxygen is as marked at first as it is later. The proportion of male-producers is approximately doubled by the oxygen.

Experiment 21. Oxygen counteracting weak manure solution. In this experiment two lines were bred continuously in weak manure solution. From the first several daughters of each generation one was selected to become the parent of the next generation. One line was reared in a dilute manure solution saturated with a 40 per cent oxygen atmosphere, the other line in a similar (but not oxygenated) solution. In the former line, only the parents and the first few daughters of each family were kept in the oxygenated solution, the other daughters being transferred to spring water. The method of conducting the

TABLE 21

Two lines of rotifers, both reared in dilute manure solution, are here recorded. One line was kept in manure solution saturated with an atmosphere of which 40 per cent was oxygen. The oxygen increased the number of male-producers

DILUTE MANURE SOLUTION, AIR				DILUTE MANURE SOLUTION, 40 PER CENT OXYGEN			
Number of generation	Date of first young	Number of ♂♀	Number of ♀♀	Number of generation	Date of first young	Number of ♂♀	Number of ♀♀
	July				*July*		
1	9	1	40	1	9	3	14
2	11	0	42	2	11	0	33
3	13	1	18	3	13	0	33
4	14	0	21	4	14	13	14
5	16	0	31	5	16	1	14
6	17	0	6	6	17	0*	3*
7	19	3	40		18	0	11
8	20	5	19	7	19	1	40
9	22	2	45	8	21	13	37
10	24	2	32	9	22	2	33
11	25	0	31	10	24	4	40
12	27	0*	23*	11	25	5	25
				12	27	1*	25*
Total		14	348			43	322
Percentage of ♂♀		3.8				11.7	

* Remainder of family not recorded.

experiment was the same as in Experiment 17, except that a manure solution was used instead of spring water. Table 21 shows that the oxygen increased the number of male-producers.

Experiment 22. Oxygen counteracting creatin. In the experiments of Shull ('11) it was shown that creatin was one of the most effective agents in the reduction of the number of male-producers. A more dilute solution of creatin was used in the following experiment. The parents alone were reared in the creatin solution. On one side of the experiment this solution was saturated with an atmosphere of which 40 per cent was oxygen. The parents on both sides were kept only 24 hours. All eggs laid in that time were allowed to hatch, and the young were reared to maturity.

Table 22, which records this experiment, shows that oxygen increased the number of male-producers.

TABLE 22

The parents of the rotifers here recorded were reared in solutions of creatin of varying concentrations; but in one-half of the experiment, the creatin solution was saturated with an atmosphere of which 40 per cent was oxygen. All offspring were reared in spring water. The oxygen increased the number of male-producers

DATE	STRENGTH OF CREATIN, IN PER CENT	CREATIN, AIR			CREATIN, 40 PER CENT OXYGEN		
		Number of parents	Number of ♂♀	Number of ♀♀	Number of parents	Number of ♂♀	Number of ♀♀
Dec.							
7	0.01	3	1	21	3	2	20
8	0.01	3	0	7	3	0	8
9	0.005	3	1	24	3	6	27
11	0.005	3	13	10	3	12	16
12	0.005	3	9	8	3	25	1
13	0.005	3	1	15	3	12	17
14	0.005	2	2	13	3	7	10
16	0.005	4	6	19	4	19	15
18	0.0083	3	0	16	3	1	10
19	0.005	4	1	15	4	2	22
20	0.0083	4	3	12	4	0	9
21	0.0083	5	1	12	5	3	22
Total.............		40	38	172	41	89	177
Percentage of ♂♀..........			18.1			33.4	

DISCUSSION

The more recent investigations upon Hydatina, conducted chiefly by Whitney and Shull, left no doubt as to the main problem which those investigations were designed to solve. External and internal factors *both* determine the amount of male-production. But the evidence of the operation of external factors was so abundant that new problems were at once created. All such agents at first discovered, and they were not few in number, had the same effect; they reduced male-production. It became important to discover the methods, or preferably method, by which these very diverse agents produced their common result; for by that means it appeared most likely that the solution of the second new problem would be reached. This latter problem was to discover a method of increasing male-production. Only in this way, it seemed, was it likely that the ultimate aim of these studies, the discovery of the physiological phenomena accompanying changes in the mode of reproduction, would be attained.

The former question, namely, that regarding the modus operandi of various chemical substances in retarding male-production, is still unanswered. The experiments described in the first pages of this paper, on osmotic pressure, acidity, and possible after effects of manure solution, led us to no conclusions. Failure to solve this problem has made the attack of the second problem, discovery of means of increasing male-production, largely a matter of trial and error. Even by this method some success has been attained; experiments 4, 5, and 6 show that calcium chloride has the desired effect upon certain lines of rotifers. Whitney's fortunate discovery that feeding the rotifers on a green flagellate increased male-production gave us our only clew. We have discussed Whitney's experiments above on pages 137 to 139, and will not repeat here.

Repeated experiments with practically uniform results have demonstrated, to our satisfaction at least, that part of the increased male-production following the use of Chlamydomonas as food, in Whitney's cultures, was due to the oxygen liberated by the green flagellates as a by-product of photosynthesis. Our results show marked effects of oxygen. However, the increase of male-production with oxygen alone is by no means as great as Whitney obtained with Chlamydomonas. We suspect, therefore, that although all the factors obviously associated with Chlamydomonas in the cultures should be separately tested before any residue of influence is assigned to nutrition, Whitney's conclusion that the food conditions influence male-production is correct, though that influence is less than he believed. We are convinced, nevertheless, that *this influence of nutrition is dependent chiefly, if not wholly, upon quality, not quantity, of food.* Although large quantities of Chlamydomonas in Whitney's cultures produced more marked results than small quantities, this is to be attributed, we think, to the greater quantity of oxygen evolved. It can not be assumed that any 'law of mass action' holds for the ingestion of food by organisms. That is, after a certain optimum (probably moderate) quantity is reached, further 'concentration' of the food does not necessarily increase the nutrition of the devouring animal. But such concentration of a green flagellate does increase the concentration of oxygen in solution. If experiments with moderate quantities of Chlamydomonas show an increase of male-production, it is probable that such increase more nearly represents the effect of nutrition itself.

With the discovery that oxygen increases male-production are we any nearer a knowledge of the fundamental causes of changes in the life cycle? We are inclined to answer in the affirmative. It may be recalled that male-production is ordinarily subject to marked periodicity (Mitchell, '13; Shull, '15). Periods of many male-producers are also usually periods of rapid growth and reproduction. Lines that yield many male-producers are also usually vigorous. Metabolic processes are going on at a rapid rate. It is hard to avoid the suspicion that in some way this speed of reaction in the protoplasm is related to the production of males. Probably not all metabolic processes, but only certain ones, are thus related to the sex-ratio. For, while, as just stated, periods of male-production are usually also periods of rapid growth, not all periods of rapid growth are accompanied by many male-producers. And while lines producing many males are usually vigorous lines, there are equally vigorous lines (judged by all known standards) that produce comparatively few males. These facts may be harmonized with the view that speed of reaction within the protoplasm makes for male-production, if we assume that only certain reactions bear this relation to sex.

If we adopt the view that the rate at which certain chemical events proceed determines the form of the life cycle, the positive results of our present experiments have some meaning. The dilute solution of calcium chloride merely provides a medium that is slightly more favorable to the processes concerned. Oxygen obviously provides for accelerated oxidation in the protoplasm. The effect of oxygen aside from male-production is not merely inferred; for the rotifers in oxygenated water, and those in Spirogyra cultures, were almost invariably healthier than the control. Sometimes the families in the oxygenated water were larger, but always the animals were more easily reared to maturity. If qualitative differences of nutrition affect the sex-ratio, as we must on the basis of Whitney's experiments assume that they do, these may likewise be conceived to affect the speed of the metabolic reactions. Even quantitative differences of nutrition, should these eventually be found to alter the sex-ratio, could conceivably alter the metabolic processes; indeed, there are no a priori grounds for believing that they do not.

The suggestion that mere speed of reaction is responsible for varying degrees of male-production becomes more plausible if its operation can be visualized in terms of known processes. It is well established, we believe, by the investigations of Shull ('12, pp. 302–308), that male-production is either caused or prevented at some time within the growth and maturation period of every parthenogenetic egg. When an egg has passed its maturation stages, the fate of the female which will hatch from that egg is sealed. She will be either a male-producer or a female-producer, according as one or another series of events has taken place in growth or maturation, and her nature is no longer subject to alteration. What happens to decide this fate is unknown. It may be the failure of some chromosome to divide. The male-producing female may have fewer chromosomes than the female-producer. The apparent variability of the number of chromosomes (Whitney, '09) may be due, not entirely to difficulty in counting them, but partly to actual differences.

If such behavior of the chromosomes results in the development of a male-producer, the rate of formation of the spindle, or of the division of chromosomes, may be the cause of the chromosome change. A chromosome dividing a little later than its fellows may be drawn (?) to one pole without completing its division. The cytology of the germ cells of Hydatina should be re-examined, with a view to discovering the difference between male-producing and female-producing females. But we emphasize that our theory of the speed of reaction is not bound up with chromosomes, to stand or fall with future discoveries regarding the chromosomes of these rotifers. Other phenomena than chromosomes which have, with present technique, no visible expression, may as conceivably be influenced by the speed of metabolic processes.

If male-production is related to the rapidity with which certain physiological processes occur, it is not surprising that a change of environment more often reduces male-production than increases it. A high degree of male-production, on our view, depends upon an efficient mechanism working at top speed. Any unskilled workman may ruin a delicate machine, it takes an inventor to improve it. Many changes of environment may retard metabolic processes, only a few accelerate them. We should expect, therefore, that most agents which affect the life cycle at all would reduce male-production, and this has been the case.

It may be suggested that our view is very near that of Whitney ('14), Mitchell ('13), and Nussbaum ('97), that nutrition is the controlling factor in the life cycle of Hydatina. If nutrition be re-defined to include all chemical processes in protoplasm, the suggested factor may be classed under nutrition. But by so defining nutrition the term metabolism becomes superfluous. Furthermore, it is clear from the context that none of the writers just named meant anything more by nutrition than quantity or quality of food devoured. We care not about the terminology, the ideas are distinct.

SUMMARY

The common effect of numerous substances upon the life cycle of Hydatina senta (diminution of male-production) is not due to their osmotic pressure, acidity or alkalinity, nor to mere delay of certain processes.

Calcium chloride, in very dilute solutions, repeatedly increased male-production in one parthenogenetic line, not in another. Magnesium chloride gave results that could not be interpreted, while potassium sulfate, iron chloride, and ammonium chloride all reduced male-production. Dilute bouillon also diminished male-production.

Oxygen in the water increases male-production. Its effect is most marked in the counteraction of agencies which diminish male-production, such as bouillon, manure solution, and creatin. Whitney's experiments with Chalamydomonas, in which male-production was greatly increased, an effect which he attributed to nutrition, are partly explained, therefore, as dependent upon the oxygen evolved in photosynthesis. Our results, however, were not as marked as Whitney's, and experiments in which the rotifers were reared with a green alga too large to be eaten, gave negative results. It is probable that nutrition has some effect, as Whitney supposed, but to what extent can not be known until the other agents which can not be eliminated are separately tested.

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