Studies on the Life-History of Protozoa.

II. The effect of stimuli on the Life-Cycle of Paramœcium caudatum.

By
G. N. Calkins and **C. C. Lieb.**

(Hierzu 5 Textfiguren.)

Stimuli of different kinds are used with such marked effects upon the human organism and undoubtedly play such an important role in the economy of nature that specific experiments upon protoplasm in less highly differentiated forms i. e. upon the single-celled organisms, cannot fail to give important results. Higher, many-celled animals are so complicated that the direct effect of stimuli upon those processes which we are wont to look upon as the „mysterious vital forces" are difficult to analyze; with unicellular animals on the other hand, these processes while still difficult of analysis, approach at least, the limits of possible explanations. Thus Paramœcium caudatum on a hay-infusion diet divides on an average, once in twenty-four hours. This rate however, is not constant, for, at times, it divides twice or more in one day, while at other times, only one division occurs during two or three days. These differences in the rate of division may be due to temperature changes, to the amount of food, or, these factors being eliminated, to the condition of the organisms which may be expressed by the term „general vitality". The effect of temperature changes or supply of food, is to induce a temporary rise or fall in the division-rate; but the effect of the condition of the organisms or general vitality is expressed quite independently of these other conditions either by a constantly decreasing division-rate, if the general vitality is running down, or by an increasing rate if the reverse. The rate of division then is a very good index of the general vitality and any foreign or novel change in the environment of Para-

mœcium that can affect the division-rate permanently, must act upon the general vitality. The effect therefore, of different substances when added to the usual food supply can be interpreted with a fair degree of accuracy, and with the division-rate as an index, the beneficial or evil influence of a chemical can be readily determined. By this means also, it may be possible ultimately, to obtain a much clearer insight into the nature of the „vital forces" than we have at the present time.

In the present paper we wish to give the general results of a few preliminary experiments of a purely tentative nature with chemical stimuli [1]) of different kinds upon Paramœcium caudatum.

It has recently been shown [2]) that Paramœcium caudatum, when fed upon the same hay-infusion diet passes through more or less regular cycles of vigor and depression, the former indicated by a higher rate of division, the latter by a constantly decreasing rate until the line ultimately dies from what Maupas [3]) described as senile degeneration. In nature it is possible that the period of depression may be ended or avoided altogether by the union of two individuals in conjugation. Such union, when successful, results in the renewal of vigor or in the „rejuvenescence" of the individuals. Conjugation therefore, like fertilization of the egg, results in the stimulation to development, or as Hertwig [4]) expresses it in „the liberation of an inhibited development", or, as we may now express it, in the renewal of the potential of vitality.

The study of the life-cycle of Paramœcium has shown that the effect of conjugation is probably analogous to that of a chemical stimulus, and further, it has led to the belief that „rejuvenescence" may be due to the presence of a new chemical compound in the form of a nucleus derived in equal parts from two individuals having had different surroundings. [5]) This supposition is supported by the fact that „rejuvenescence" can be brought about in a weakened Paramœcium by chemical stimuli, the chemical in this case taking the place of the other half nucleus. In the following pages we shall describe some further experiments in this direction.

---

[1]) The term „stimulus" is used throughout in its general sense and not in the way defined by Loeb, 1900.

[2]) G. N. Calkins, Studies on the Life-History of Protozoa. I. The Life-Cycle of Paramœcium caudatum. Archiv f. Entw. 1902.

[3]) Arch. d. Zoolog. Expérim. et Générale. 1889. (2), VII, pp. 149–517.

[4]) Abh. d. K. bayr. Akad. d. Wiss. München. II. Kl. XIX pp. 1–104.

[5]) Calkins loc. cit.

I. The effect of artificial stimuli upon weakened Paramœcium.

In the first of these Studies, it was shown that Paramœcium at the low period of a cycle may be revived to new activity by simply changing the medium from hay-infusion to beef-extract.[1]) This was done repeatedly until the number of generations far exceeded what may be considered the ordinary life cycle (about 170 generations).[2]) In all probability the effect of the beef-extract is not due to the small percentage of proteid matter contained in it, nor to the bacteria which develope there, but, as Liebig long since pointed out, probably to the extractives from the beef which it contains. Some of the most important of these are the so-called „earthy phosphates" including salts of potassium, sodium and magnesium. Up to the present time only potassium phosphate (K_2HPO_4) has been used with Paramœcium in periods of depression.

A. Experiments with potassium phosphate.

At intervals throughout the year isolated individuals of Paramœcium have been treated with potassium phosphate. The animals were placed in a solution of 1 pt. to 1000 of the di-basic salt for variable periods, the longest not exceeding one hour and twenty minutes. In every case where treatment was given at periods of depression, the result was a marked increase in the rate of division and continued life, whereas the sister-cells, continued on hay-infusion, invariably died. The following experiment gives a correct illustration of the action of this salt upon the general vitality of the organism.

March 20th, one of the daughter-cells of A_2,[3]) was treated for 30 minutes with potassium phosphate diluted 1 to 1000. Another daughter-cell was treated with beef-extract at the same time. At this period the race as a whole was on a decline and the lines A_3 and A_4, fed exclusively on hay-infusion, died out shortly after. A_1 and A_2 had been stimulated with beef-extract on the 28th of February, and were not as weak as their sister-cells A_3 and A_4 which had not had beef-extract since December 15th.

---

[1]) Calkins loc. cit.

[2]) At the present time (June 1902) the A series has reached the 605th and the B series the 568th generation.

[3]) A_2 is one of the four lines of Paramœcium (A_1 A_2 A_3 A_4) kept in culture since the original A was isolated in February 1901. There are four lines of B also (B_1 B_2 B_3 B_4) decendants of the original B isolated Feb. 1, 1901.

The immediate effect of the salt was to cause a rapid backward movement across the slide, then the animal righted itself and moved swiftly around the cell in which it was confined. This lasted for about three minutes after which it became quiet and behaved in a normal manner. At the expiration of a half hour the same performance was repeated when the Paramœcium was transferred to hay-infusion. On the following day it had divided once and twice again on the day after. The comparative rate of division can be seen very clearly in Diagram 1. The number of divisions per day are averaged for seven-day periods.

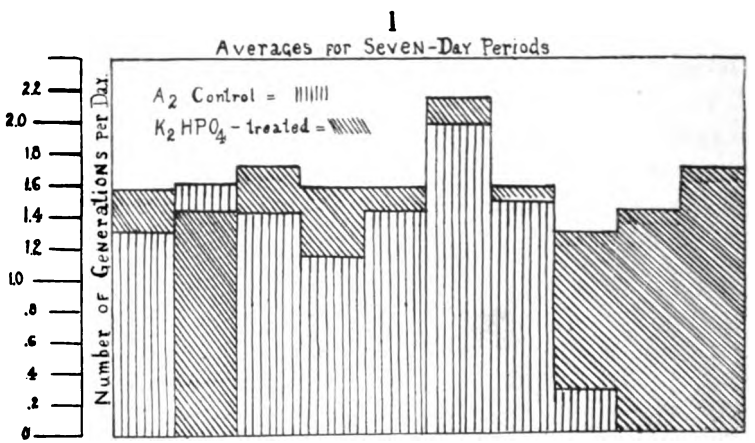

Diagram I.

Two sister-cells, one of which was treated for 30 minutes with K_2HPO_4, while the other was treated as usual with hay-infusion. In only one period does the latter exceed the former.

This diagram indicates that not only does the chemically-stimulated form divide more often than the beef-stimulated one, but it also shows that fluctuations are less noticeable. The beef-fed A_2 line died out on the 12th of May after 69 generations while the other one at that period was in its 88th generation and is now (June 1st) living in the 117th without showing any evidence of depression.

II. The effect of stimuli in preventing periods of depression and in sustaining the general vitality.

Here we have to do with stimuli which are applied at regular intervals while in the preceding case we dealt with an initial stimulus

which was not renewed. The stimuli used have been of many different kinds but we shall limit the description here to experiments with beef-extract, alcohol and strychnine.

A. The effect of beef-extract applied at regular intervals.

From December to the middle of April certain lines (A_1 A_2 B_1 B_2) of Paramœcium were stimulated regularly (that is once per week for

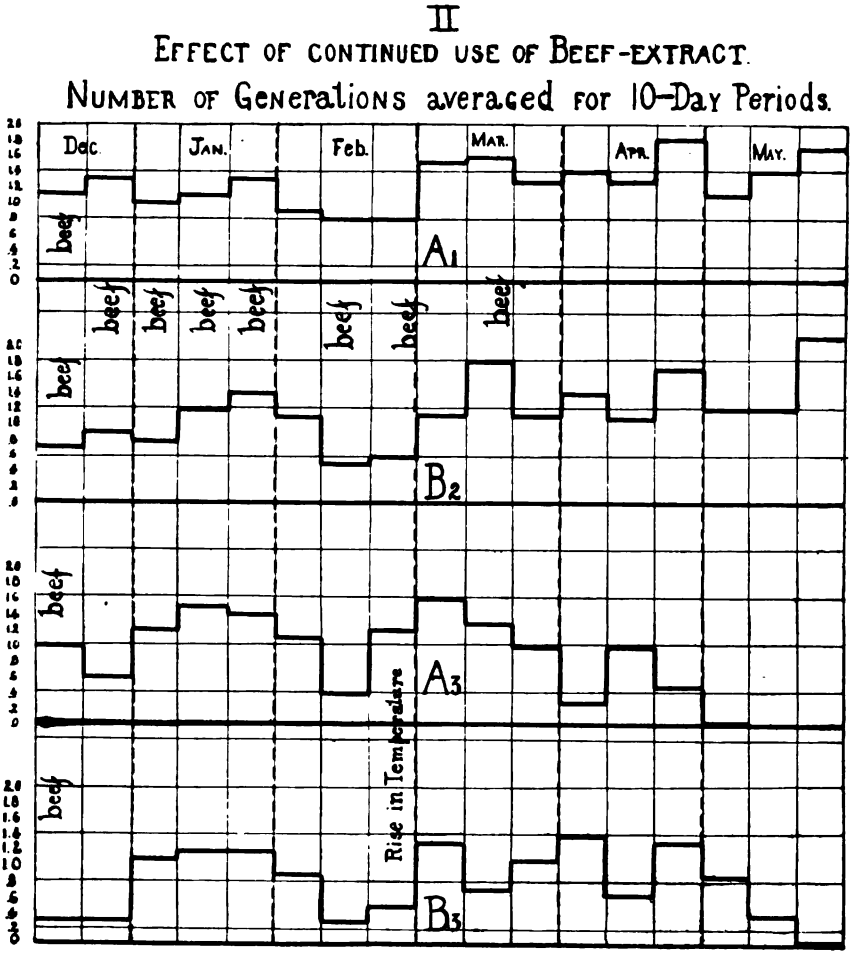

Diagram II.

Two lines of Paramœcium caudatum, A_1 and B_2, were treated at weekly intervals with beef-extract; while two sister-cells were continued on hay-infusion (A_3 and B_3).

twenty-four hours) with beef-extract while the other lines (A_3 A_4 and B_3 B_4) after an initial stimulus in December, were fed constantly upon hay-infusion. The following curves show the history of the general vitality of each, much more clearly than would a description. The hay-fed A_3 and A_4 died out May 5th after 139 and 108 generations; the hay-fed B_3 and B_4 lines died out on the 24th of May after 138 and 164 generations respectively. All lines of beef-fed individuals are still living.

Beef-extract is not a food in the ordinary sense and Paramœcium does not divide as rapidly in it as in hay-infusion. The effect of the use of beef upon A_1 A_2 and B_1 B_2 may be seen in the fact that the total numbers of generations of the beef-fed lines at first fell behind those of the hay-fed ones. The more rapid division of the hay-fed forms and the total number of generations of the beef-fed lines soon overtook and passed those of the hay-fed. The following table illustrates this important fact for the A series (A_1 beef-fed once per week, A_3 hay-fed):

Date	Total number of Generations		Difference between A_1 and A_3
	A_1 (Beef)	A_3 (Hay)	
Dec. 15th	379	379	0
„ 24th	389	389	0
Jan. 6th	404	404	0
„ 15th	417	419	2—
„ 25th	431	435	4—
Febr. 5th	440	447	7—
„ 15th	449	463	14—
„ 25th	457	466	9—
March 7th	473	476	3—
„ 17th	488	491	3—
„ 27th	501	505	4—
April 7th	516	519	3—
„ 10th	520	521	1—
„ 15th	526	526	0
„ 18th	530	528	2+
„ 28th	547	540	7+
„ 3rd	554	542	12+

Both A_1 and A_3 were stimulated with beef on Decembsr 15th. The stimulus was repeated at weekly intervals upon A_1 until the latter part of March. A_3 was not stimulated again. Both were given the same food at the same time except during the periods of stimulation. The effect of the initial stimulus of A_3 is seen in the period of vigor when the total number of generations increased more rapidly than for A_1 which was retarded at stated intervals by the

beef-extract. Notwithstanding this retardation, the general vitality of A_1 continued to be far stronger than that of A_3 and although the weekly stimulus was stopped at the end of March, the potential of vitality of A_1 was so much greater than that of A_3 at the same time, that when the latter died out (stock and all decendants of A_3 died May 6th) the former was dividing at a high rate which still continues (June 1st).

The same results were obtained with the B series under similar treatment (See curves for B_2 beef-fed, and B_3 hay-fed).

The experiment indicates that an initial stimulus with beef-extract (and the same explanation applies to the effect of potassium phosphate) imparts to the organism some potential power which cannot be derived from the usual food, and by which the metabolic activities are enabled to continue for a more or less definite period, after which, if the stimulus is not renewed, the activities become more and more feeble and the individuals die from what Maupas called „old age". Old age or the sinking of the general vitality in these forms at least can thus be prevented by a very simple expedient.

Not only beef-extract with its salts, but other substances as well are capable of bringing about similar results. Thus alcohol, which some observers regard as a food, others as merely a stimulus in the sense used above, will enable the organisms to maintain a high rate of division throughout periods of depression of sister-cells not thus stimulated. Strychnine also has the same general effect. The results are given in more detail below.

B. The Effect of Continued Stimulation with Alcohol. [1])

For these experiments solutions of alcohol of different strength were used; and a variable number of drops of each strength were added to the usual hay-infusion medium, thus ensuring the regular food and a stimulant at the same time. This was repeated every day. For convenience the experiments may be grouped in series, according to the strength of the alcohol. The following table gives a general review of the results.

---

[1]) The following experiments were worked out entirely by C. C. Lieb upon Paramœcium from G. N. Calkins' cultures.

Table I.

Date		Control. No Stimulus. B_2	Series I. 1 pt absolute Alcohol to 10000 of Water				Series II. 1 pt absolute Alcohol to 1000 of Water				Series III. 1 pt absolute Alcohol to 500 of Water			
			Hay 4 pt Alcohol 1 "	Hay 3 pt Alcohol 2 "	Hay 2 pt Alcohol 3 "	Hay 1 pt Alcohol 4 "	Hay 4 pt Alcohol 1 "	Hay 3 pt Alcohol 2 "	Hay 2 pt Alcohol 3 "	Hay 1 pt Alcohol 4 "	Hay 4 pt Alcohol 1 "	Hay 3 pt Alcohol 2 "	Hay 2 pt Alcohol 3 "	Hay 1 pt Alcohol 4 "
October	5	1	1	1	1	1								
"	7	2	4	4	4	2								
"	8	4	6	5	5	3								
"	9	6	8	7	7	5								
"	10	7	10	9	8	6								
"	11	8	13	12	11	8								
"	12	10	15	14	12	10								
"	14	13	17	17	15	12								
"	15	14	19	19	17	15								
"	16	16	21	21	18	16								
"	17	18	23	23	20	18								
"	18	20	25	24	21	19								
"	19	22	26	25	23	21								
"	21	23	28	27	26	23								
"	22	24	29	28	27	24								
"	23	26	30	29	29	25								
"	24	27	32	31	31	27								
"	25	29	33	32	32	28								
"	26	30												
"	31	32					1	1	1	1				
November	1	34					3	3	3	2				
"	2	36					5	5	5	3				
"	4	38					7	8	8	4				
"	5	39					9	10	10	5	1	1	1	1
"	6	40					10	10	11	6	2	2	2	2
"	7	41					11	11	13	8	4	4	5	4
"	8	42					12	13	15	10	6	6	7	6
"	9	44					14	15	17	12	8	8	9	8
"	10	47					16	17	19	15	12	11	12	11
"	12	48					17	17	21	16	15	12	14	13
"	13	50					19	18	22	17	16	13	15	14
"	14	51					21	18	24	19	18	15	16	15
"	15	52					23	18	26	20	20	16	18	17
"	17	54					26	18	30	23	23		22	20
"	18	55					27	18	32	24	24		22	21
"	19	56					29	18	32	25	26		24	22
"	20	57					31	18	33	27	27		26	24
"	21	58					32	18	35	28	29		27	26
"	22	59					34	18	36	30	31		29	27
"	25	62					36		40	33	35		33	30
"	26	64					38		42	34	36		34	31
"	27	65					39		43		37			
"	28	66					40		45		39			
"	29						41		45		40			
December	1						44		48		43			
"	2						45		49		44			
"	3						46		51		47			
"	5								54		49			
"	6								55		50			
"	7								57		52			
"	9								60		55			

Table I.

Date	Control. No Stimulus. B_2	Series I. 1 pt absolute Alcohol to 10000 of Water								Series II. 1 pt absolute Alcohol to 1000 of Water								Series III. 1 pt absolute Alcohol to 500 of Water							
		Hay 4 pt	Alcohol 1 „	Hay 3 pt	Alcohol 2 „	Hay 2 pt	Alcohol 3 „	Hay 1 pt	Alcohol 4 „	Hay 4 pt	Alcohol 1 „	Hay 3 pt	Alcohol 2 „	Hay 2 pt	Alcohol 3 „	Hay 1 pt	Alcohol 4 „	Hay 4 pt	Alcohol 1 „	Hay 3 pt	Alcohol 2 „	Hay 2 pt	Alcohol 3 „	Hay 1 pt	Alcohol 4 „
December 12														63				58							
„ 14														65				60							
„ 16														67				63							
„ 18														70				66							
„ 20														72				68							
„ 23														75				71							
„ 25														78				74							
„ 28														82				78							
„ 30														84				80							
January 1														86				83							
„ 3														88				85							
„ 4														89				86							
„ 6														90				88							
„ 8														92				90							
„ 11														94				93							
„ 14														97				95							
„ 16														100				97							
„ 18														102				99							
„ 20														105				101							
„ 22														108				103							
„ 24														111				107							
„ 26														112				108							
„ 27														114				111							
„ 30														117				114							
February 1														118				116							
„ 3														121				119							
„ 5														123				122							
„ 7														124				125							
„ 8														125				126							
„ 10														125				127							
„ 12														128				130							
„ 14														130				133							
„ 15														132				134							
„ 17														134				137							
„ 20																		141							
„ 23																		145							
„ 25																		148							
„ 27																		150							
March 1																		153							
„ 3																		156							
„ 5																		159							
„ 7																		162							
„ 8																		163							
„ 10																		164							
„ 12																		167							
„ 15																		169							
„ 17																		171							
„ 19																		174							
„ 21																		176							
„ 24																		179							

This table indicates that alcohol has no effect when taken in too weak doses (series I) and too powerful an effect when taken in overstrong doses (series III). It further indicates, however, that when a medium dose is given (for example 3 pts. of $^1/_{1000}$ to 2 of hay or 1 pt. $^1/_{500}$ to 4 parts of hay) the effect is a continued stimulus which sustains the high rate of division even during periods of depression of the control series (series II). The effect can more readily be seen with the aid of diagrams.

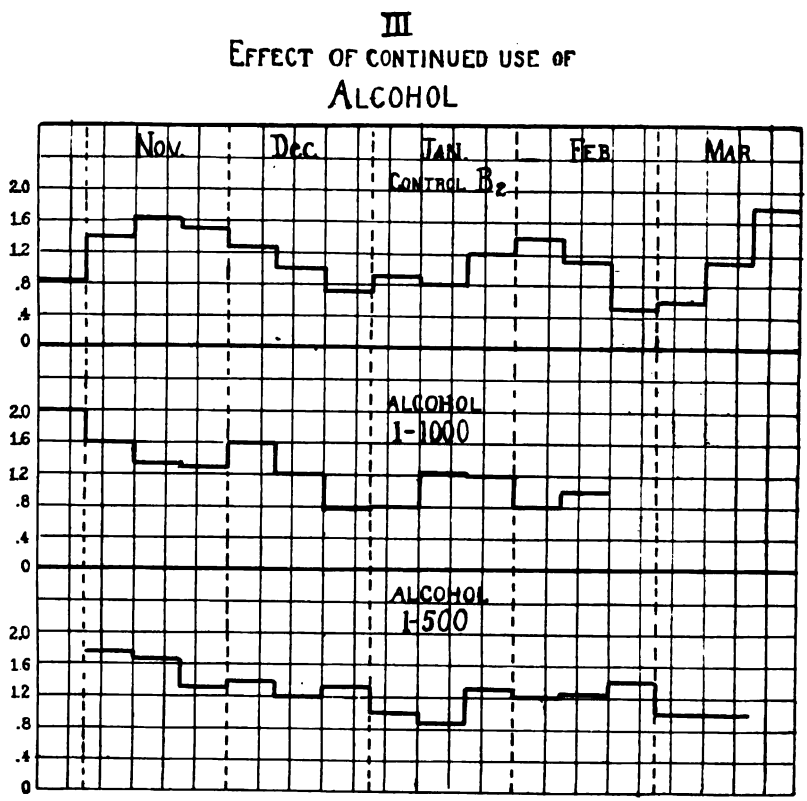

Diagram III.

Two sister-individuals were treated with alcohol of different strength; one with one part to one thousand; the other with one part to five hundred. Another individual was carried along at the same time upon hay-infusion (control). The one in the weaker alcohol died near the middle of February; the other alcohol-treated one was discontinued after the middle of March.

Analysis of these curves shows an interesting fact, viz. that there is the same tendency to regular decrease of the division-rate

as in the control, indicating that the underlying factor, — decreasing vitality, — is operating in each case, but the stimulated forms receive something from the alcohol which enables them to live at a more rapid rate; (Series $^1/_{1000}$ [see diagram] increased at a rate of 15 % higher than A, while series $^1/_{500}$ went 33 % higher). Thus, during the December period of depression, in the regular series Control B_2, the hay-fed individuals all died and the line was continued with some individuals that had been stimulated with beef-extract about the middle of December; the same depression, however, is noted in the curves representing the stimulated individuals, although the period of depression does not end in death. In the case of one of the experiments, (1—500) the depression continued a month after recovery of the control and its recovery did not take place until the control was nearly on the verge of a second period of depression (Feb.). This recovery however, was not perfect and the division-rate never again rose to the height reached after the initial stimulus. The experiments were discontinued in March and it remains to be shown whether the continued use of alcohol of this strength, will, like the beef-extract, keep up the general vitality indefinitely. There is no doubt that for a time at least, alcohol will prevent death during periods of depression, whether it acts like the beef-extract cannot be stated with certainty. From these curves, there is evidence to show that it does not, and that the general vitality would decrease under the constant stimulus as it does under the treatment with hay-infusion alone, although much more slowly.

There is no doubt that the presence of alcohol in the food of Paramœcium makes these organisms far more lively than when in hay-infusion alone, and this combined with the higher rate of increase, indicates much more rapid metabolic processes. Notwithstanding the more rapid living, the general vitality does not seem to be affected badly by the alcohol. To test this point, some of the alcohol-treated Paramœcium were kept in clear hay-infusion without alcohol. The results are given in the following table on page 366.

While the control Paramœcium ran down in the February period of depression, the others retained the usual rate of division and showed no signs of decreasing vitality. From this result it may be concluded that the alcohol exacts no physiological usury during the period of treatment, but it cannot be inferred from these experiments alone, that alcohol like beef-extract, restores the high potential of vitality. Further experiments, carried out for much longer periods, must be undertaken before this point can be finally determined.

Date	Control B_2		Alcohol 1—1000		Alcohol 1—500	
	Generations	Division rate	Generations	Division rate	Generations	Division rate
January 23	1		1		1	
„ 27	7	1,4	8	1,6	5	1,0
February 1	13	1,2	13	1,0	10	1,0
„ 6	19	1,2	20	1,3	17	1,3
„ 11	23	0,8	24	0,9	21	0,9
„ 16	26	0,6	30	1,3	28	1,4
„ 21	27	0,2	37	1,3	34	1,1
„ 26	29	0,4	43	1,1	39	1,0

1—1000 was one from Series II.

1—500 was from Series III.

Only enough of the records are given to show the general results.

Comp. Diagram III.

C. The effect of continued stimulation with Strychnine.

Experiments with strychnine were conducted in the same way as those with alcohol. The control was one of the A_2 series, fed constantly (except when necessary for recovery during periods of depression) with hay-infusion. The stimulant was used in various proportions; in Series I. there was one part of strychnine to 30000 parts of water, and in Series II. there was 1 to 40000. As with alcohol the stimulant was given daily. The following table gives the records in condensed form:

Table II.

Date	Control. No Stimulus A_1	Series I. 1 pt Strychnine to 30000 of Water				Series II. 1 pt Strychnine to 40000 of Water			
		Hay 4 pt Strych. 1 „	Hay 3 pt Strych. 2 „	Hay 2 pt Strych. 3 „	Hay 1 pt Strych. 4 „	Hay 4 pt Strych. 1 „	Hay 3 pt Strych. 2 „	Hay 2 pt Strych. 3 „	Hay 1 pt Strych. 4 „
November 28	1	1	1	1	1	1	1	1	1
„ 29	2	2	2	2	1	2	2	2	1
December 1	5	5	5	4	3	5	5	5	3
„ 2	5	6	6	5	3	5	6	6	3
„ 3	6	8	7	6	4	8	8	8	4
„ 5	8	9	9	8	5	10	9	9	4
„ 6	8	10	10	8	5	13	10	10	4
„ 7	8	12	12	10	6	13	12	12	4
„ 9	10	12	13	11	7	15	15	15	4
„ 12	11	14	16	14	9	19	19	19	4
„ 14	11	18	18	15	10	21	21	22	6
„ 16	12	22	20	17	12	24	23	24	7
„ 18	13	23	21	18	13	26	25	26	10
„ 20	13	24	21	18	14	27	27	29	12
„ 23	14	27	21	19	15	30	30	31	14
„ 25	15	30	22	21	15	33	32	33	15

Date		Control. No Stimulus A_1	Series I. 1 pt Strychnine to 30000 of Water				Series II. 1 pt Strychnine to 40000 of Warer			
			Hay 4 pt Strych. 1 "	Hay 3 pt Strych. 2 "	Hay 2 pt Strych. 3 "	Hay 1 pt Strych. 4 "	Hay 4 pt Strych. 1 "	Hay 3 pt Strych. 2 "	Hay 2 pt Strych. 3 "	Hay 1 pt Strych. 4 "
December	28	19	34	22	22	16	36	36	35	17
"	30	20	35	22	23	17	37	37	37	19
January	1	22	37	22	25	17	40	40	40	21
"	3	24	39	22	27	19	42	42	42	23
"	4	25	39		28	20	43	43	43	24
"	6	27	40		30	21	44	45	45	26
"	8	29	43		32	23	47	47	47	29
"	11	33	46		34	24	51	50	50	30
"	14	35	48		36	25	54	52	52	33
"	16	37	50		37	26	55	53	53	35
"	18	40	52		39	28	58	56	55	37
"	20	43	55		41	29	61	59	58	40
"	22	46	58		43	30	63	62	60	41
"	24	49	60		45	31	66	63	62	43
"	25	51	61		47	32	67	65	63	44
"	27	53	63		47	33	69	67	65	46
"	30	56	66		48	34	72	70	66	47
February	1	58	68		50	36	74	72	68	48
"	3	60	70		51	37	76	74	70	50
"	5	62	72		53	38	79	76	71	51
"	7	65	75		55	39	80	77	72	53
"	8	66	76		56	40	82	79	74	54
"	10	66	77		57	41	83	80	75	55
"	12	68	79		59	42	84	81	76	57
"	14	70	81		60	43	87	84		59
"	15	71	82		61	44	90	86		60
"	17	73	85		63	46	91	87		62
"	20	76	88		65	46	94	90		63
"	23	79	91		68	48	97	92		65
"	25	80	93		69	48	100	94		65
"	27	80	95		71	49	102	96		66
March	1	80	97		73	49	105	98		67
"	3	81	99		74	50	108	101		68
"	5	83	101		75	50	109	103		69
"	7	84	102		76	51	112	104		69
"	8	85	103		76	51	114	105		70
"	10	88	104		77	52	115	107		72
"	12	90	106		77	52	117	109		73
"	15	93	106		77	54	119	112		75
"	17	95	107		78	54	123	114		76
"	19	96	109		78	55	125	116		77
"	21	98	108		79	55	127	117		78
"	24	101	108		79	56	129	120		

With the stronger solutions of strychnine the general effect was to reduce the rate of division. This may be due, in part at least but not entirely, to the exclusion of the normal food medium (e. g. 4 parts of strychnine solution to one of hay-infusion leaves compa-

ratively little food). (See diagrams.) With weaker solutions of the drug, e. g. one part in 40 000, the effect is more striking. (See diagram IV.) The division-rate on the whole is higher than that for the control and the periods of depression so characteristic of the regular control series (Dec. and Feb.) are conspicuous by their absence. The entire curve is very regular.

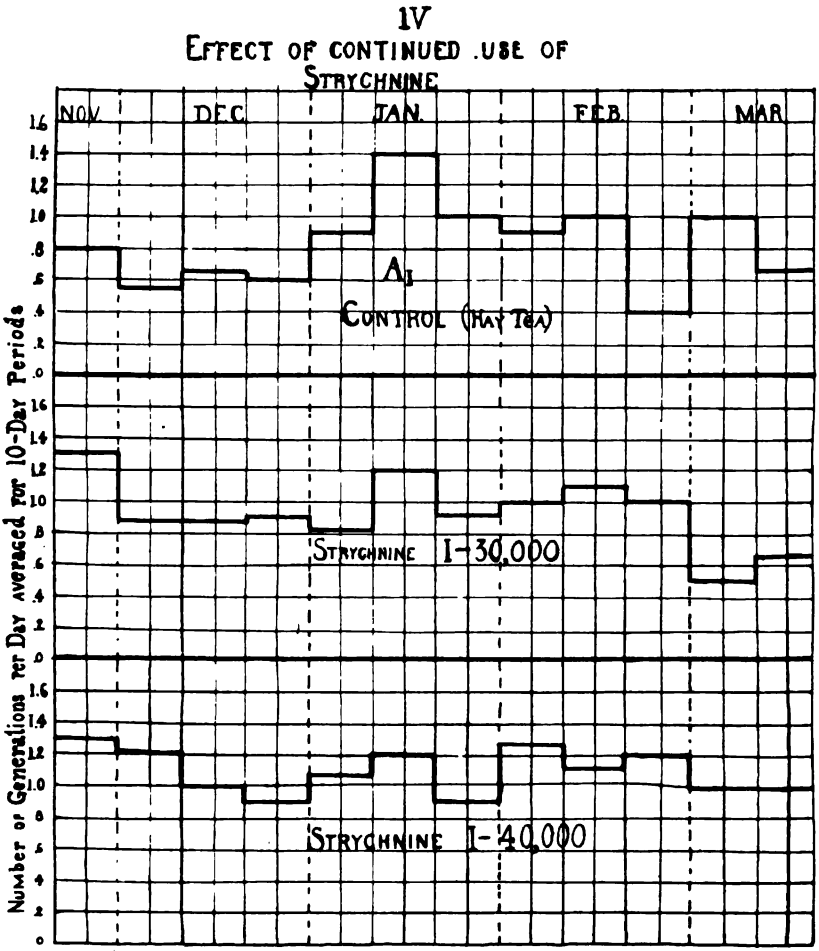

Diagram IV.

Two sister individuals were treated daily with strychnine of different strength; one part in thirty thousand parts of water; and one part in forty thousand parts of water. Both showed an initial stimulus and a more regular divison-rate than the control which was continued on hay-infusion.

The stimulus is not lasting, that is, there is no renewal of vitality after an initial stimulation with strychnine as there is, for example, after beef-extract. Individuals if removed from the strychnine medium to clear hay-infusion, rapidly decrease in the rate of their division and soon die. This is shown by the following diagram.

V

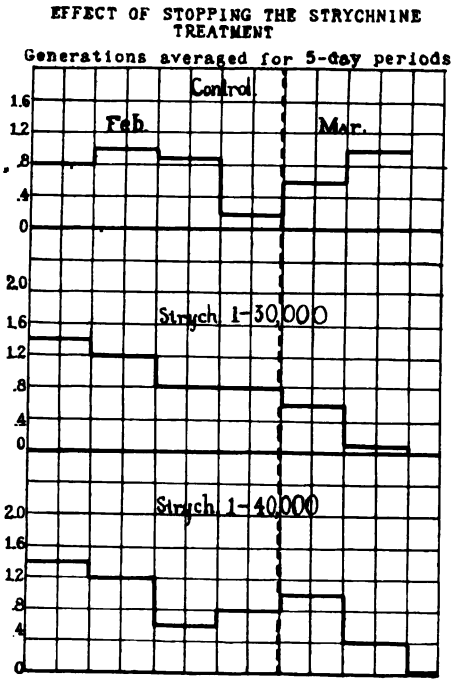

Diagram V.

One individual from each set of the strychnine experiments was given fresh hay-infusion daily without strychnine. The result in each case was a more or less rapid decrease in the division-rate and final death, while the sister-cells, treated continuously with strychnine, maintained the high rate of division (see Diagram IV).

It may be pointed out here that during the period represented above, the sister-cell that had been continued in the strychnine medium, did not exhibit the same decrease and final death. (Cf. Diagram IV.)

There is undoubtedly some action in Paramœcium induced by strychnine. When first immersed in it the organisms show considerable irritability and dart about the slide in a very restless manner. This ceases after a short time and the organisms look and act like the normal. In the division-rate there is an invariable initial stimulus after which it becomes fairly regular and uniform with the control save for the fact that the characteristic periods of depression are omitted. After the stimulant is stopped and the organisms are placed in the hay-infusion, the division-rate rapidly falls and the individuals soon die. We cannot clearly interpret this result at the present time. If, as Loeb (1901) supposes, there is a specific mortal process at work in living organisms, which can be check ed by the use of certain drugs, then we might suggest that strychnine has such an effect on Paramœcium. It certainly does not act like beef-extract, nor indeed, like alcohol, for there is

no renewal of vitality nor increase in constructive metabolic activity, and when the stimulant is withdrawn the animals die. Like the effect of potassium cyanide on sea-urchin eggs, its effect appears to be to prevent or to postpone death, but this conclusion is expressed only as an assumption.

General Conclusions.

The „vital forces" of Paramoecium caudatum, especially those involved in the functions of general metabolism, growth and reproduction, are subject to periodic sequences of vigor and depression. A couple of hundred generations, more or less, uses up the potential of vitality, whereupon, unless the potential is renewed, the race dies out with some indications of protoplasmic old age. During the cycle, however, the single mass of protoplasm of the original organism by virtue of these „vital forces" is capable of growing and dividing again and again until the progeny might reach an inconceivable number (2 to the 170th power) and tons upon tons of protoplasm might be formed. The potential for all of this is apparently bound up in the one minute ancestral cell and becomes weakened as the generations multiply. The organism may be compared with a storage battery which gradually runs down as its charge its used. Like the battery, the infusorian cell is capable of being re-charged and of inaugurating a new cycle of generations.

One natural way in which the weakened descendant may be restored to new vigor is by conjugation with another individual (of the same or of a different race) which has had a different environment.[1])

Another way is by stimulation with chemicals. The experiments described above show that an initial treatment with beef-extract will completely restore the vitality of weakened forms; also, that a simple mineral salt acting only for 30 minutes, has a similar effect.

Other experiments with alcohol, strychnine, etc., show that, upon continued stimulation the tendency to become worn out is partly overcome and the „sinking of the life activities" is prevented. The effect of the alcohol appears to be different from that of strychnine and both effects are different from that of the potassium salt. The latter appears to act upon the organism as a whole,[2]) i. e. upon the „general

---

[1]) cf. Studies etc., I.

[2]) There is considerable morphological evidence to show that this chemical affects the nucleus directly. G. N. C.

vitality"; the alcohol, apparently, as in man, upon the secreting activities, while the strychnine seems to perform a negative role by, possibly, preventing oxidative-processes and postponing death. If these interpretations are correct we must distinguish between the secreting activities, those that underlie all activities and which may expressed by the „general vitality"; and again those that are expressed by destructive metabolism. The above experiments appear to indicate that each of these may be reached more or less independently of the others by stimuli of different kinds, and all such stimuli appear to affect the entire organism and to prolong its life.

Another conclusion which may be drawn from these experiments is that, in all probability, Paramœcium is not entirely dependent upon conjugation for renewal of vitality. In nature the environment of these forms must be constantly changing; every rain-fall washes new materials into the ponds and pools, and not only new food material, but with it salts of various kinds. Even a slight trace of sodium chloride, magnesium chloride, potassium phosphate or chloride is sufficient to renew the activity of weakened forms.[1]) It is a question furthermore whether the organisms are ever in a weakened state in nature. The artificial conditions of the laboratory are undoubtedly just suited to a limited cycle of generations, while stimuli, introduced periodically, prevent it. In nature, with a more or less constant change in environment, it is not at all improbable that Paramœcium retains a fairly regular division-rate, varying only with the temperature and amount of food and only slightly because of changes in the „general vitality".

[1]) Experiments with these salts have been tried in the laboratory and all give positive results.

Columbia University New York City, June 1, 1902.