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LIGHT REACTIONS AND METABOLISM IN MAY-FLY NYMPHS

I. REVERSALS OF PHOTOTAXIS AND THE RESISTANCE TO POTASSIUM CYANIDE

II. REVERSALS OF PHOTOTAXIS AND CARBON DIOXIDE PRODUCTION

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FOUR CHARTS

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I. REVERSALS OF PHOTOTAXIS AND THE RESISTANCE TO POTASSIUM CYANIDE[1]

HISTORICAL

The idea that there is a relation between the fundamental metabolic processes and the signs of the reaction of animals to stimuli is not new. It has also occurred to some investigators that there may be a relation between the rate of these metabolic processes or part of them and the phototactic reaction. Holmes ('05) found that Ranatra is made more positive by conditions that cause an increase in activities and made more negative by opposite conditions. Carpenter ('05), studying the light reactions of Drosophila, concluded that the more stimulated the animals were, the more positive they became.

Jackson ('10) came to the conclusion that the changes in responses to light, which he obtained with the amphipod Hyalella, are not due to chemical changes of the eye or skin, as Loeb ('10) had suggested, but are rather due to a sudden stimulation or shock to the nervous system.

Mast ('11, p. 283) states that in Arenicola, "Any condition which serves as a depressant tends to cause the young larvae to become negative," and he concludes (p. 287) that "The facts 1) that the light reaction may be affected in a given organism by so many contrasting conditions; 2) that the same change in external conditions may cause opposite reactions indifferent organisms, and 3) that the sense of the reaction may be changed without any immediate external change—indicate that these responses are due not to a direct and specific effect of the environment on some definite chemical compound within the organism, but rather to the effect on the organism as a whole."

Bohn ('12) separately reached a somewhat similar conclusion when he decided that there are two kinds of sensibility, one to light and one to shade, and that these correspond to antagonistic

[1] The experiments upon which part 1 of this paper is based were performed at Williams College in 1913–14. Certain tests were repeated by the junior author in the spring of 1915. The nymphs were identified by Mr. W. A. Clemens, of the Cornell Limnological Laboratory, to whom we express our thanks.

chemical reactions. Causes that accelerate oxidations tend to make animals positive to light and causes that inhibit oxidations produce reactions to shade. Bohn's conclusions are based on his own and Drzewina's ('11) observations.

Phipps ('15) found that when amphipods, negative to light, are treated with potassium cyanide, chloretone, or were subjected to decreased oxygen tension or to starvation that many of the animals reversed their reactions both to light intensity and to the direction of rays.

None of these workers measured the effect of the substances upon the metabolism of the animals under investigation. Indeed, this has been done only by MacCurdy ('13), who found that the negative starfish Asterias forbesi gives off less carbon dioxide in sunlight than in shade. He made no effort to control the reaction to light.

Many investigators, beginning with Loeb ('04), have recorded reversals of sign of the phototactic response which could be produced at will; the problem which we set for ourselves was to find whether or not there is any correlation between the sign of the reaction to light and the rate of metabolism of the animal as measured by resistance to potassium cyanide or by carbon dioxide production. For the first part of this inquiry the May-fly nymphs Leptophlebia sp? and Epeorus humeralis (Morgan) were chosen because of their abundance near the laboratory and because Wodsedalek ('11) had found the phototactic reactions of the May-fly nymph Heptagenia interpunctata is readily reversed by chemicals.

ECOLOGICAL NOTES

The Leptophlebia and Epeorus nymphs studied lived in mountain streams with stony beds and rapid currents such as are quite common in the Berkshire Hills. Their distribution was most carefully studied in Tunnel Brook (near Hoosac Tunnel). In the early autumn when few leaves had fallen the nymphs lived on the under sides of stone and were almost invariably facing upstream. Their clinging ability enabled them to maintain them-

selves in the swift current. In April and May large numbers of Leptophlebia were found among submerged leaves in the quieter parts of the brook. At the same time Epeorus was most abundant under stones in more rapidly moving water. Neither were found on the upper surface of stones until late in May when the adults were emerging in large numbers. At this time hundreds of Epeorus nymphs could be seen on the upper side of any large stone in the brook, all headed against the current.

METHODS

The phototactic tests were made in a large, light-tight wooden box to which light was admitted through adjustable slits. The opening was near the bottom of the box so that the light entered the end of the experimental dishes. During the experiments described in the first part of this report a north window furnished the light source. The experimental box was painted dead black inside. The rear was curtained with a heavy black, cloth. A semicircular opening in the floor of the box opposite the light inlet permitted the observer to sit with head and upper body inside the box without introducing an appreciable amount of light.

The light-reaction tests were carried on in oblong glass dishes measuring 12 x 5.2 x 2.2 cm., which were placed with the long axes parallel with the light rays. When it was necessary to cool the dishes to keep the temperature at or below tap temperature, the experimental dishes were placed in shallow glass trays and packed in ice on all sides except that toward the window. Control dishes were kept under identical conditions save for the factor under experimentation.

The nymphs were kept in the laboratory in aluminum tea balls which hung in running tap water which was similar in salt and gas content to the water of their native streams. Most of the experiments were performed before the animals had been in the laboratory five days.

REVERSALS OF PHOTOTAXIS

In experiments upon phototaxis the nymphs were selected from a number which had stood a short time in tap water exposed to the light conditions under which the test was to be made. Then, if the experiments were to be upon negative animals, only decidedly negative nymphs were selected. During the course of the experiments nymphs were subjected to various strengths of sulphuric, hydrochloric, and acetic acids; potassium and sodium hydroxide; potassium, sodium, calcium and magnesium chlorides; ethyl alcohol, chloretone, caffein, strychnine, and to temperature changes.

The two species with which we worked reacted oppositely to light. Epeorus was normally positive while Leptophlebia was normally negative. The former is the more definite in its reaction, as shown by the ratios of the average negative (N) and positive (P) responses of the controls. Thus Epeorus with seventeen sets of control readings gave a $\frac{P}{N}$ ratio of $\frac{90}{11}$ while Leptophlebia with thirty such controls gave $\frac{5}{12}$.

Quantitative reversals were obtained with Epeorus with ethyl alcohol, calcium chloride, and with a decrease of temperature. Alcohol was the most efficient reversing agent used with this species. At tap temperature (about 12°C.) the best results were obtained with a 2 per cent solution. When the temperature was lowered 5° or more, better results were obtained with a 1 per cent solution. Under both conditions quantitative reversals were obtained with positive nymphs and with the control strongly positive throughout.

With Leptophlebia more of the chemicals tried gave quantitative reversals. Hydrochloric and sulphuric acids, potassium and sodium hydroxides, potassium cyanide, and potassium chloride gave 100 per cent reversals of negative nymphs while the controls were predominantly negative throughout. Sodium and calcium chlorides gave 80 per cent reversals with the controls over 80 per cent negative. Magnesium chloride, chloretone, and caffein had little effect. Alcohol, as with the other species, gave a high percentage of reversals, although quantitative results were rare.

TABLE 1

Showing the results of tests as to whether 0.00001 normal solutions of potassium cyanide directly measures the metabolic rate of Epeorus nymphs and 0 000001 N. indirectly measures it. Also as to whether 0.0025 N. directly measures the rate of metabolism of Leptophlebia. Division A of the table exhibits results on animals that should have theoretically the lower rate of metabolism

STRENGTH OF KCN	NUMBER TESTED	SIZE LIMITS	AVERAGE SIZE	TEMPERATURE	STIMULATED OR UNSTIMULATED	SURVIVAL TIME	NUMBER TESTED	SIZE LIMITS	AVERAGE SIZE	TEMPERATURE	STIMULATED OR UNSTIMULATED	SURVIVAL TIME	RATIO OF DIFFERENCE TO PROPABLE ERROR	SUPPORT EXPECTATION	STATISTICALLY SIGNIFICANT
	Division A						Division B								
Epeorus															
		mm.	*mm.*	*deg. C.*		*minutes*		*mm.*	*mm.*	*dec. C.*		*minutes*			
0.00001 N	42	6.0–9.0	6.9	As B.	⅋	153 ± 8	50	3.0–5.5	4.4	See A	⅋	111 ± 6	3.0	*	*
0.00001 N	17	3.5–8.0	6.0	As B.	⅋	163 ± 12	19	4.0–8.0	5.7	See A	&	92 ± 4	4.3	*	*
0.00001 N	8	3.5–7.0	5.9	7	⅋	247 ± 20	8	4.0–8.5	5.6	13	⅋	148 ± 16	2.8	*	*
0.000001 N	19	6.0–11.0	8.5	As B.	⅋	48.5 ± 4.3 hrs	10	3.3–5.0	4.6	See A	⅋	97.7 ± 9.4hrs	3.6	*	*
Leptophlebia															
0.0025 N	4	8.0–8.5	8.3	As B.	⅋	79 ± 6	5	7.0–8.5	7.8	See A	&	49 ± 0.6	4.5	*	*
0.0025 N	85	6.0–8.5	6.8	As B.	⅋	231 ± 7	15	3.0–5.5	4.9	See A	⅋	169 ± 10	3.6	*	*
0.0025 N	49	6.0–11.0	7.6	2–4	⅋	559 ± 13	85	6.0–8.5	6.8	11	⅋	231 ± 7	16.4	*	*
0.0025 N	6	7.5–8.5	8.3	0–1	⅋	366 ± 22	4	8.0–8.5	8.3	17–21	⅋	79 ± 6	10.0	*	*

⅋ Indicates that the animals were not mechanically stimulated.

& Indicates that they were mechanically stimulated to actively move.

DOES RESISTANCE TO POTASSIUM CYANIDE MEASURE THE RATE OF METABOLISM IN MAY-FLY NYMPHS?

In this work the cyanide resistance method of Child ('13) was used without modification except in the strength of the cyanide solutions employed. In this method with a relatively high concentration the animals with a higher rate of metabolism (cf. Geppert, '99, Hyman, '16) die before those with a lower rate. In much weaker solutions acclimatization occurs and the effect is reversed. The resistance of the nymphs was tested in 500-cc. Erlenmeyer flasks. Control experiments showed that the more sensitive Epeorus nymphs could live from five to fifteen days in this amount of unchanged tap water, provided the temperature was approximately constant.

Some difficulties were encountered in determining the death point. The nymphs were usually observed every thirty minutes in later experiments every twenty minutes), and those that were apparently motionless were removed with a large pipette and carefully inspected under a lens. If no motion was apparent they were stimulated with a needle. If they failed to show any motion under these conditions they were considered dead. It is obvious that this treatment would stimulate live nymphs and more frequent inspection would increase rather than decrease the experimental error.

Epeorus proved to be much less resistant to the cyanide than Leptophlebia. With the former (table 1) a solution 0.00001 normal gave a measurement of metabolism by the 'direct method;' with the latter the same measurement was obtained by a strength of 0.0025 normal. With Epeorus 0.000001 normal was found to measure metabolism by the acclimatization method. With this dilution so great care was necessary in order to maintain the solution at even its approximate strength that no serious tests were run.

A new solution of 0.1 normal potassium cyanide was made up weekly, and from this dilutions were made to the desired strength. With extreme dilutions and with certain preliminary experiments which ran several days the solutions were made up fresh twice daily.

The inquiry as to whether resistance to potassium cyanide in May-fly nymphs is affected by the rate of metabolism of the animal was prosecuted along the following lines:

1) What is the relation between the resistance of large (old) and small (young) nymphs?

2) What is the effect of stimulation upon the resistance to the cyanide?

3) What is the effect of differences in temperature?

The results of these experiments are summarized in table 1. Nymphs of Epeorus were less resistant to 0.00001 normal potassium cyanide when they were small, or stimulated, or at increased temperature. All of these conditions cause a higher rate of metabolism (Child, '13; Allee, '14). On the other hand, in a 0.000001 normal solution the smaller (younger) nymphs were more resistant than the larger ones, which is what the theory demands if a solution of this strength to measure indirectly the rate of metabolic processes.

Leptophlebia in 0.0025 normal solution was less resistant when young, or stimulated, or when the temperature was increased so that this strength of cyanide directly measures the metabolic rate of these nymphs. A solution 0.00025 did not indirectly measure the rate of metabolic processes of these nymphs and the experiments were not continued long enough to find a solution strength that would do so. In all the above instances in which the evidence is that cyanide resistance does measure the rate of metabolism the differences in the survival times exceeds twice the probable error and we may safely hold them to be statistically significant.

RELATION BETWEEN THE SIGN OF LIGHT REACTION AND RESISTANCE TO THE CYANIDE

1. Epeorus

The average survival time of fifty-one positive Epeorus nymphs (table 2) which had been taken directly from tap water was 108 ± 5 minutes. These nymphs averaged 5.4 mm. long. A reversal of the phototactic reaction of forty-seven other posi-

tive Epeorus nymphs was caused by treatment with alcohol (1 or 2 per cent) and decreased temperature (2° to 8°C.). These reversed nymphs gave a survival time of 131 ± 6 minutes in the same strength of cyanide. Their size average was 6.5 mm. The difference in survival time is only 2.1 times the probable error, and since the difference may have been affected by the difference in size, too much emphasis cannot be laid on these results.

The survival time of eighteen nymphs of the same species that failed to reverse in the alcohol-reduced temperature treatment was 88 ± 3 minutes. Their average length was 6.1 mm. The nymphs that were reversed by this treatment lived forty-three minutes longer in the cyanide than those that remained

TABLE 2

Showing the relation between the sign of the phototactic reaction of Epeorus nymphs and their resistance to potassium cyanide

NUMBER TESTED	AVERAGE LENGTH	AVERAGE SURVIVAL TIME	TREATMENT BEFORE KILLING	NUMBER TESTED	AVERAGE LENGTH	AVERAGE SURVIVAL TIME
Positive nymphs				Negative nymphs		
	mm.	*minutes*			*mm.*	*minutes*
51	5.4	108 ± 5	Tap water			
18	6.1	88 ± 3	Alcohol	47	6.5	131± 6
			Lowered temperature	11	6.1	160±16

positive. Since this is 4.8 times the probable error, it must be significant.

The majority of the Epeorus nymphs treated with alcohol and decreased temperature reversed their reaction to light and had a slightly (perhaps questionably) lower rate of metabolism than the control animals and a decidedly lower rate than those not reversed by the treatment. The nymphs that remained positive, although given the alcohol-low temperature treatment, apparently had been stimulated by the alcohol, for they showed a higher rate of metabolism than the control animals. This can be explained by assuming that the alcohol acted as usual, first stimulating and later depressing. If this be true, the fact that alcohol plus reduced temperature was more effective than the

latter alone becomes significant if the metabolic differences prove to be causal rather than incidental or resultant, for the difference between a stimulated period followed by depression may well be greater than mere depression.

Sometimes quantitative reversals were obtained by reducing the temperature. Eleven nymphs that had been thus reversed (average length 6.1 mm.) were killed in cyanide and gave an average resistance of 160 ± 16 minutes. This is fifty-two minutes longer than that given by the control nymphs which is 2.5 times the probable error and is probably significant.

Taken altogether, the evidence here presented indicates that reversed Epeorus nymphs have a lower rate of metabolic activity than do positive animals. One other observation confirms this idea. Epeorus nymphs collected in October were kept in a large aquarium that also contained some fresh-water mussels. In December and January the nymphs were found to be dying in large numbers. When tested all were negative to light, although when first collected they had given almost quantitatively positive reactions. Obviously the metabolic process of the nymphs was strongly retarded and this is correlated with their reversal to light.

2. Leptophlebia

Leptophlebia nymphs were usually negative in their reaction to light giving a ratio of twelve negative to five positive animals. The average survival time of sixty-one untreated nymphs (average length 7.9 mm.) that gave the usual negative light reaction was 131 minutes. Forty-two positive nymphs (average length 7.8 mm.) under conditions similar in every way resisted the same strength of cyanide 130 minutes. Thus there was no difference in the metabolic condition of these two groups that could be measured by the cyanide resistance method.

Hydrochloric acid was very effective in causing reversals in Leptophlebia. The survival time of thirty-six nymphs so reversed (average length 7.7 mm.) was 127 minutes. Twenty nymphs similarly treated that remained negative (average length 7.8 mm.) gave a mean survival time of 120 minutes. This

difference is of course negligible as is also the difference between these acid-treated animals and the control.

Alcohol was also effective in making these negative nymphs positive. Fourteen nymphs that were made positive gave a resistance of 91 ± 7 minutes to the cyanide as compared with 130 ± 4 minutes for the sixty-one control animals. This is 3.5 times the probable error. The eleven nymphs that were treated with alcohol and remained negative showed some stimulation

TABLE 3

Showing the sign of phototactic reaction of Leptophlebia nymphs and their resistance to cyanide

NUMBER TESTED	AVERAGE LENGTH	AVERAGE SURVIVAL TIME	TREATMENT BEFORE KILLING	NUMBER TESTED	AVERAGE LENGTH	AVERAGE SURVIVAL TIME
Positive nymphs				Negative nymphs		
	mm.	*minutes*			*mm.*	*minutes*
36	7.7	127	HCl	20	7.8	120
14	7.8	91 ± 7	Alcohol 2 per cent	11	7.7	109 ± 9
9	7.8	119	H_2SO_4			
8	7.8	115	Acetic acid	2	7.0	136
7	8.5	127	NaOH	8	7.8	146
8	6.9	132	KOH	2	7.0	130
9	6.8	133	KCN	2	7.4	103
3	8.3	115	KCl	2	8.0	103
6	8.7	100	NaCl	4	8.7	105
			MgCl	2	8.0	129
2	7.8	129	$CaCl_2$	3	7.8	123
6	7.8	161	Chloretone	8	8.1	165
108	7.8	123	Totals and averages	64	7.8	129
42	7.9	131 ± 4	Tap water control	61	7.8	130 ± 4

when tested with cyanide, but not so much as the positive nymphs.

Considering the numbers tested and the small deviation from the resistance shown by the control animals, none of the results obtained with other reagents and listed in table 3 are significant with the exception of those with chloretone. This drug was not very efficient in causing reversals, but did cause decided depression, as measured by the cyanide method, and caused reversals in about 30 per cent of the nymphs treated.

The average effect of all these reagents upon the resistance to cyanide, if such is worth anything, shows no marked difference between positive and negative treated animals nor between the treated animals and the control.

From these experiments with Leptophlebia we have the interesting results that these nymphs were reversed without affecting their resistance to potassium cyanide (HCl and averaged results); with accompanying stimulation (alcohol) and with accompanying depression (chloretone).

The quantitative reversal of positive Epeorus nymphs and negative Leptophlebia by 1 per cent alcohol in the same experimental dish at the same time was repeatedly demonstrated. Thus conditions absolutely identical caused opposite reversals in the two species. At first sight this would appear to mean that both positive and negative animals were reversed for the same reason. This is not necessarily true. In the tests to find the strength of potassium cyanide that would directly measure the metabolic rate of the two species it was found that Leptophlebia was only one-fourth as sensitive as Epeorus. Since the Leptophlebia are much more resistant, a strength of alcohol that only stimulated them may have clearly depressed Epeorus. That such is the true explanation is indicated by the effect of alcohol on the resistance of the two species of nymphs to cyanide. The Epeorus that had been made negative were found to be depressed, while the Leptophlebia that were made positive were clearly stimulated.

II. REVERSALS OF PHOTOTAXIS AND CARBON DIOXIDE PRODUCTION[2,3]

METHODS

The experiments upon which the second part of this report is based were carried on at Lake Forest College upon a May-fly

[2] This section is based on experiments by the senior author, now being continued, which were started in the spring of 1916. They were made possible by money grants from the Elizabeth Thompson Fund and from the Bache Fund of the National Academy.

[3] The nymph whose reactions are described in the second part of this paper is *Heptagenia pulchella* Walsh. We are indebted to Professor J. G. Needham for this identification.

nymph belonging to the Heptageninae. These nymphs are quite common in Pettibone Creek (Shelford, '13, maps) where they are usually found in the stones between riffles. Pettibone Creek is a brook about the same size as the Berkshire streams mentioned in the preceding part, but with much less rapid current.

The nymphs were kept in the laboratory for long intervals during the winter in well aerated running-water aquaria. Animals to be experimented on were transferred to room-temperature aquaria aerated by means of an air-pressure pump operated by water pressure.

The experiments were conducted as at Williamstown save that a daylight, concentrated filament, 100-watt Mazda (C2 of the General Electrical Company) was used as a source light. The experimental box was placed in a darkened room so that only light from the source lamp could enter during the experiment.

In most of this work an assistant plotted the reactions of individual nymphs to light and manipulated their change to experimental solutions. Careful controls were run. When the nymphs were to be changed to a new experimental solution, the controls were changed in the same manner to fresh tap water. The assistant also prepared the pairs of nymphs for their carbon dioxide test in such a way that the experimenter had no idea which was the experimental and which the control nymph.

The carbon dioxide production was determined in Tashiro's biometer (Tashiro, '13, '17) as follows: The assistant placed two nymphs, whose rate of carbon dioxide production was to be compared, momentarily on filter paper and then transferred each to a shallow glass cell.

The nymphs used were of the same size and were selected so that the experimental factor was the only known cause for variation in their rate of carbon dioxide production. The containers were marked for future identification. These were handed to the experimenter and immediately placed in the apparatus. Within five minutes from their removal from the experimental dishes one could get an indication of their relative rate of carbon dioxide production. Under optimum conditions the entire process of determination could be repeated at the rate of three per hour. This was much in excess of the usual rate.

TABLE 4

9/1/16. *1:20 p.m. Put two lots of eight May-fly nymphs each in aquarium water in dark box. Light: 60-watt Mazda, 50 cm. distant. The nymphs had been in the laboratory two days. Temperature aquarium 21.5; of room 22.*

	LOT 1					LOT 2				
	+				−	+				−
1:30					8	1		2		5
1:32	1		1		6	2	1		5	
1:48	1				7	2				6
1:53		2		1	5				8	
2:01			3		5	1	1			6
2:06		1		2	5	1		2		5
2:07		1	1	1	5		1	1		6
2:08		1	1	1	5			1		7
2:09	1	1			6			1		7
2:10	1	1			6			1		7
2:11		2		1	5			2		6
2:12	1		1		6			2		6
2:13	1		2		5			2		6
2:14		1	1		6			1		7
2:15			2		6			1		7
2:18	1*				7*			1		7

* Positive one has been positive throughout put in biometer 2:20 with a negative one that has been negative all the time.

Washed with CO_2 free air 5 minutes.
Positive nymph in left chamber, negative in right.
Bubble 2:25.
First ppt. Rt. 2:28.
Much more left 2:38.

Took out biometer, 2:40. Both active; put back in dark box in separate dishes.

3:00 Both reacting as before testing in biometer.
3:20 Do.
3:45 Do.
4:10 Do.
4:15 Replaced in biometer as before.
4:25 Bubble.
4:27 First left, positive.
4:40 More on left as before.
4:45 Out of apparatus. Reintroduced at negative end of separate dishes.
5:00 Nymph that had been positive throughout, still positive; other negative as before.
6:00 Both negative.
7:40 Nymph negative throughout now positive, other negative. Light on all night.
8:00 a.m. Both negative.

Some idea of the nature of experimentation together with its effect on the nymphs may be gained from table 4. This table is a slightly expanded copy of a portion of the laboratory record for the day. It shows that during almost four hours of observation, in which time three biometer tests were made, the nymphs maintained their original light reaction and that each test showed the positive nymph had the higher rate of carbon dioxide production.

Whatever the faults of the method used, it at least has the merit of being always comparative. As was to be expected, large nymphs gave off carbon dioxide more rapidly than small ones and active nymphs more rapidly than inactive ones. These nymphs are strongly thigmotactic, and this usually caused them to remain quietly in their container. Occasionally one would move. Such determinations were of course thrown out.

PHOTOTAXIS AND CARBON DIOXIDE PRODUCTION IN UNTREATED NYMPHS

These nymphs, like Leptophlebia, are usually negative to light. This normal reaction to light is graphically shown in chart 1 together with the preliminary and control records shown in charts 2, 3 and 4.

About 20 per cent of the untreated nymphs were positive to light. When these were tested they were found to have a higher rate of carbon dioxide production than the negative nymphs. Their $\frac{P}{N}$ ratio, based on 332 non-selected control readings was 5.5 This is indicated by the graphs in columns 1 and 2 of chart 1 and the details are given in table 5.

Exposure to light for considerable time sometimes caused negative nymphs to reverse their light reaction and become positive. Such animals were found to be more stimulated, as determined by the rate of carbon dioxide production, than similar nymphs that had not been exposed to light (table 6). This is just the opposite to the result obtained by MacCurdy with negative starfish which he found gave off less carbon dioxide when exposed to strong light.

Chart 1

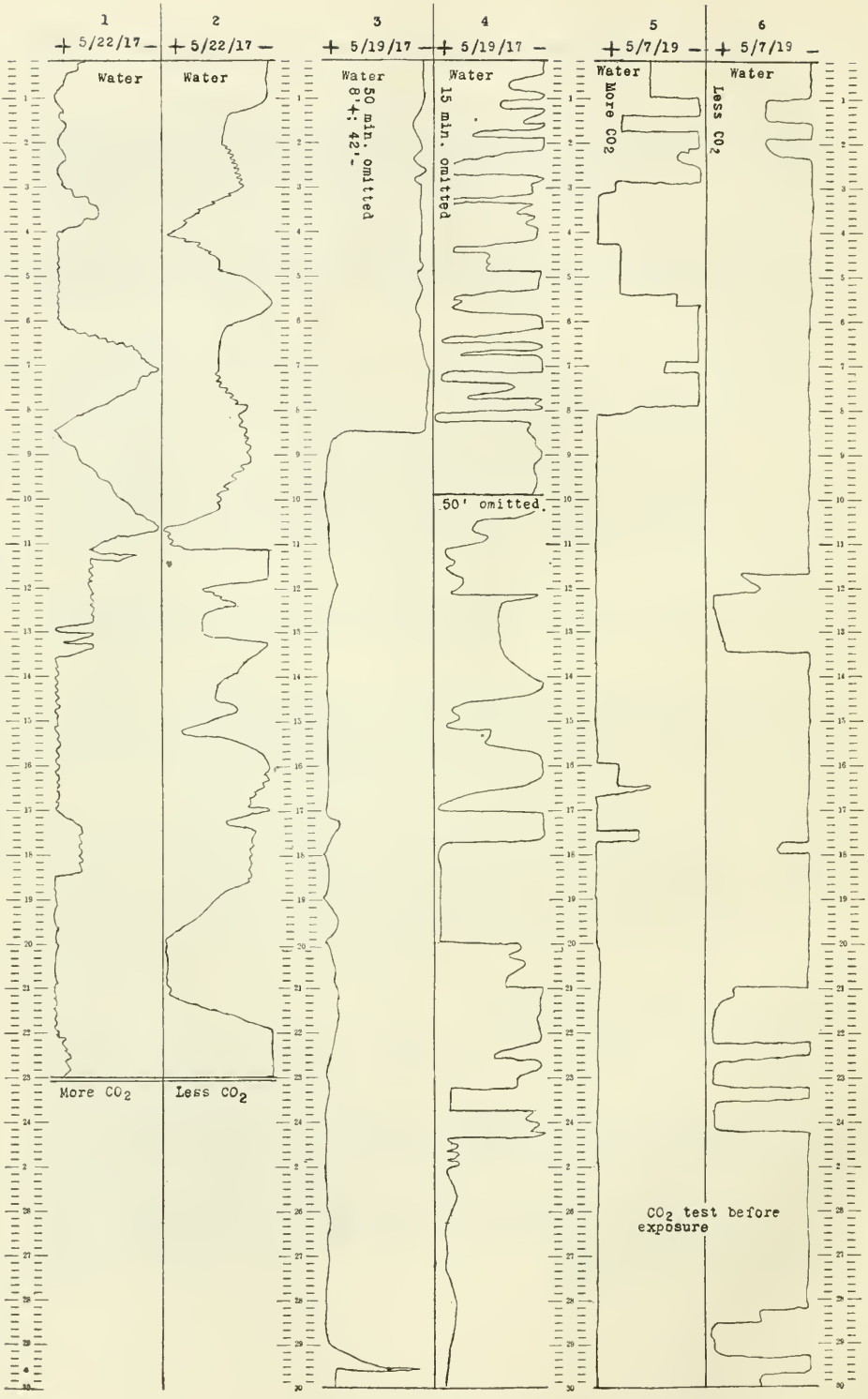

1
+ 5/22/17 −
Water
More CO_2
2
+ 5/22/17 −
Water
Less CO_2
3
+ 5/19/17 −
Water
50 min. omitted
8'+: 42'-
4
+ 5/19/17 −
Water
15 min. omitted
50' omitted.
5
+ 5/7/19 −
Water
More CO_2
CO_2 test before exposure
6
+ 5/7/19 −
Water
Less CO_2

TABLE 5

Showing the comparative rate of carbon dioxide production of positive and negative nymphs under control conditions (p. 525)

DATE	SIZE		MORE CO_2
	+	−	
	mm.	*mm.*	
9/ 5/16	8.0	8.0	Positive
9/ 1/16	6.0	6.5	Positive
	6.0	6.5	Positive
9/ 2/16	5.5	6.0	Positive
	5.5	5.5	Positive
10/26/16	7.0	7.0	Positive
10/27/16	7.5	8.0	Positive
10/28/17	7.1	7.0	Positive
	7.0	7.0	Negative. More active before test
	8.0	8.0	Negative. More active before test
11/ 2/16	6.5	7.0	Positive
3/12/17	11.5	12.5	Positive
	11.5	12.5	Positive
3/13/17	11.5	11.0	Positive
	11.0	11.0	Positive
	11.0	11.0	Positive
	11.0	11.0	Positive
	11.0	11.0	Positive. Not marked. Demonstrated
3/15/17	10.0	10.0	Positive
3/16/17	10.0	10.0	Positive
3/20/17	11.0	11.0	Positive
3/21/17	11.0	11.0	Positive
3/22/17	10.0	10.0	Positive

Number tested 23 pairs. Positive more, 21. Negative more, 2.

Chart 1 Showing graphically the reaction of six untreated nymphs to light from a 100-watt daylight Mazda (C2) placed 50 cm. from the experimental dishes. The charting was done by an assistant from whose records these graphs were copied. The scales show time in minutes. The left-hand side represents the positive end of the dish, i.e., the end toward the light. Where the line is approximately straight vertically the nymph was resting quietly. Curves and kinks show movement which did not markedly change the position in the dish in respect to light. Column 1 gives the reactions of a nymph that was predominantly positive to light, which after twenty-three minutes' exposure gave more carbon dioxide in the biometer than the negative nymph whose reactions are recorded in column 2. Column 3 shows a nymph made positive by long exposure to light. In this case the reversal came suddenly. Column 4 gives the same result obtained by a different method. Perhaps column 3 represents a 'tropic' and column 4 a 'trial' reaction. Columns 5 and 6 show the reactions of two nymphs that were tested in the biometer before exposure to light. The animal with the higher rate of carbon dioxide production became positive.

TABLE 6

Showing the effect of long exposure to light upon carbon dioxide production

SIZE		TEMPERATURE		MINUTES EXPOSED	MORE CARBON DIOXIDE
Exposed	Unexposed	Exposed	Unexposed		
mm.	*mm.*	*deg C.*	*deg C.*		
13	13	21	19	120	Exposed
13	14	21	19	150	Exposed
10	10	23	21	204	Exposed
10	10	23	21	234	Exposed
10	10	15	13	52	Exposed

EXPERIMENTS WITH HYDROCHLORIC ACID

1. Effect on negative nymphs

As with the other species studied, hydrochloric acid was one of the most effective reagents in causing reversals. In one set of carefully controlled, fully plotted experiments there were a total of 220 control or preliminary readings lasting from fifteen to ninety-three minutes. Of the nymphs thus tested, thirty-seven were or became positive without other treatment than exposure to light. This is 17 per cent of the number tested.

In this same series of experiments 125 nymphs that had been consistently negative through a preliminary testing period of at least fifteen minutes, were treated with N/25 hydrochloric acid. Under this treatment, seventy-five nymphs, 60 per cent, became positive. Of the fifty nymphs that did not reverse, twenty-five were kept under observation for less than twenty-five minutes and only two were kept until they died. If it had been the purpose of the experiments to ascertain how many nymphs could be reversed by the treatment, doubtless about 90 per cent would have become positive before death resulted.

The typical effect of the acid upon the light reactions of these nymphs is shown in chart 2. The graphs show that the nymphs may reverse soon after being placed in the acid or the reversal may come only after long exposure. Forty-eight per cent of the reversals came within fifteen minutes, but reversals occurred after seventy minutes' treatment. Death frequently followed close upon the later type of reversals.

The effect of this treatment upon the carbon dioxide production is shown in table 7, which lists all the determinations, and in table 8, which partially analyzes the results listed in the preceding table.

The biometer tests show that the nymphs were stimulated when first put into the acid and that this period of stimulation lasted approximately fifteen minutes. The time limits varied with different individuals. After this period of stimulation the nymphs were depressed. This gives two periods in the carbon dioxide production corresponding to the two periods in the reversals by this strength of the acid.

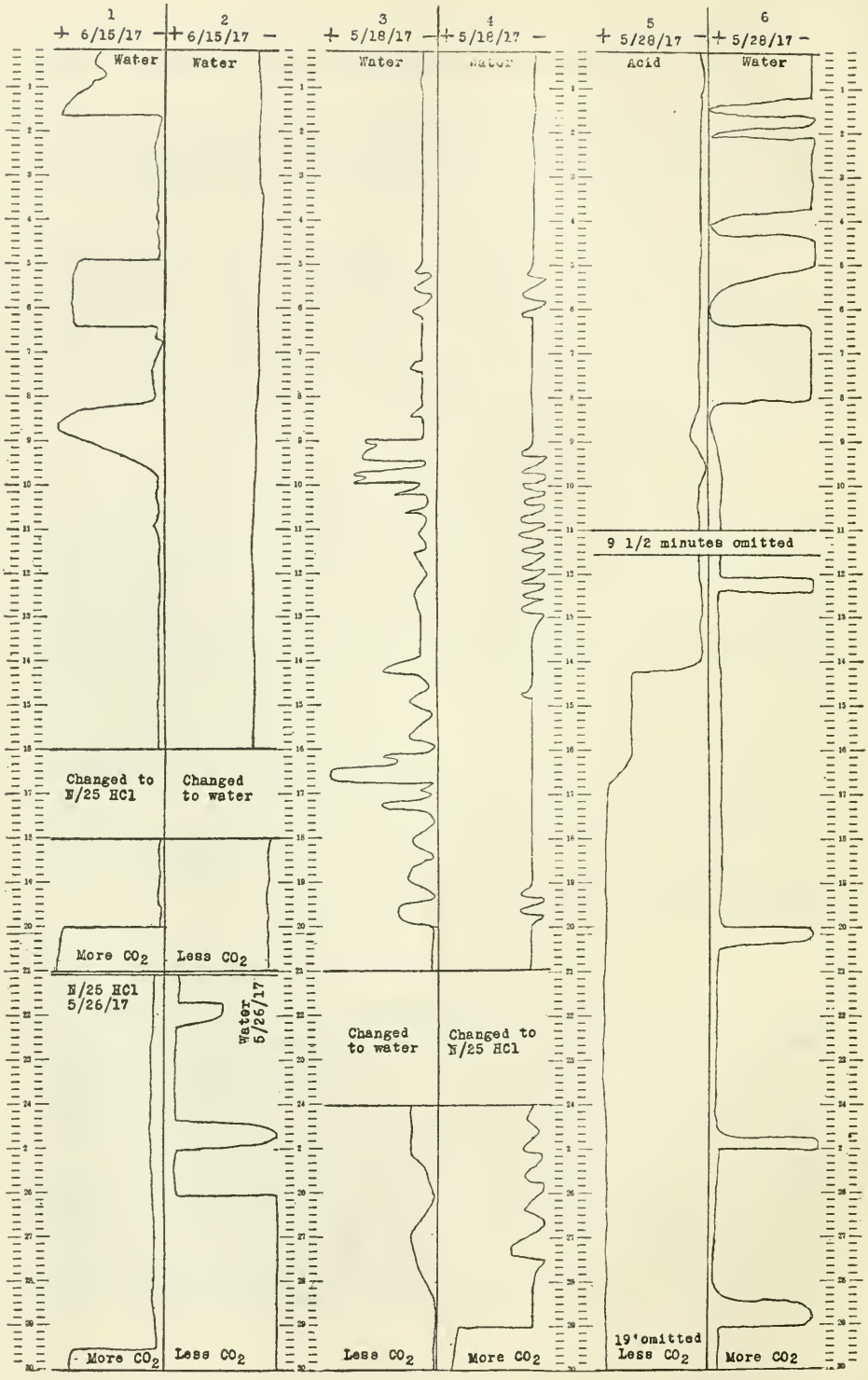
The tables show that all nymphs were not tested immediately after reversal, but of those whose carbon dioxide production was found within two minutes after reversal and which had been stimulated by the acid the average time of treatment was 13.2 minutes. Thirteen of the twenty-five nymphs so tested had reversed within ten minutes after being placed in the hydrochloric acid. On the other hand, the average time of treatment of the animals similarly reversed and measured, but giving more carbon dioxide in the control than in the acid, was twenty-seven minutes, and seven of the seventeen nymphs reversed after twenty-seven to thirty-seven minutes' treatment.

From these experiments it appears that either a marked increase or a decrease may accompany phototactic revérsals of these nymphs when treated with hydrochloric acid.

2. Effect on positive nymphs

About 20 per cent of the Heptageninae tested were positive to light when first tested or became positive under the influence of exposure to light for a short time. This positive reaction is much less stable than the usual negative reaction. Of fifteen carefully plotted tests lasting from 15 to 114 minutes, seven, or 47 per cent, showed a change to the usual negative reaction without treatment. In nineteen tests lasting from 1 to 68 minutes seventeen of the nymphs, 89 per cent, were made negative by N/25 hydrochloric acid. Most of these became negative within the first five minutes of treatment and, as was to be expected from the preceding

Chart 2


1
+ 6/15/17 −
Water
Changed to N/25 HCl
More CO_2
N/25 HCl 5/26/17
More CO_2
2
+ 6/15/17 −
Water
Changed to water
Less CO_2
Water 5/26/17
Less CO_2
3
+ 5/18/17 −
Water
Changed to water
Less CO_2
4
+ 5/18/17 −
Water
Changed to N/25 HCl
More CO_2
5
+ 5/28/17 −
Acid
9 1/2 minutes omitted
19' omitted
Less CO_2
6
+ 5/28/17 −
Water
More CO_2

experiments, they were found to be stimulated by the treatment (table 9).

Two instances of the reversal of positive animals by acid treatment are given in the first two columns of chart 4, p. 450. In both instances shown the reversals are typical in that they are very marked and occur almost directly upon the start of acid treatment. In the second instance shown the experimental dish was twice turned end for end and each time the nymph moved directly negative.

Here we have the same reagent making negative animals positive by either stimulating or depressing them and making positive animals of the same species negative by stimulating them.

3. Negative vs. positive nymphs; both treated with HCl

The treatment with hydrochloric acid did not cause all the nymphs to reverse their light reactions. When animals that had been made positive were compared with those still negative, it was found (table 10) that the former had a higher rate of carbon dioxide production when the time of exposure had been approximately the same for both nymphs compared. Almost all the nymphs tested came from the preliminary period of stimulation. Theoretically, nymphs long exposed to acid and positive would give less carbon dioxide than those more recently placed in the acid and still negative. This possibility was not tested, since the results can easily be interpolated from the tests given in previous tables.

Chart 2 Showing graphically the light reactions of four nymphs treated with N/25 HCl and their carbon dioxide production as compared with that of their control nymphs. Column 1 shows two reversals with the acid treatment, the first of which occurred within three minutes. The reversals shown in column 1 and that in column 4 were accompanied by stimulation. On the other hand, the reversal shown in column 5, which came after twenty-three minutes' exposure to the acid and which was allowed to remain positive for thirty-five minutes before testing, showed depression. The preliminary test in columns 5 and 6 is not charted, but was essentially like the first eight minutes shown. It will be noted that in this test the control became positive due to exposure to light and was therefore more stimulated than the ordinary negative control. Other tests show depression by treatment with acid for this length of time when compared with negative nymphs.

TABLE 7

Showing the effect of N/25 hydrochloric acid upon carbon dioxide production. All the nymphs here listed were decidedly negative in their light reaction before being placed in the acid

SIZE		SIGN OF LIGHT REACTION		MORE CO_2	MINUTES IN HCl	MINUTES BETWEEN REVERSAL AND TEST
+	−	HCl	Control			
13	13	+	−	Acid	2	1
13	13	+	+	Acid	2	1
11	11	+	−	Acid	2	1
13	13	+	−	Acid	3	1
13	13	+	−	Acid	5	2
13	13	+	−	Acid	5	2
12	12	+	−	Water	5	2
11	11	+	−	Water	6	1
10	10	+	−	Acid	6	1
10	10	+	−	Acid	6	1
11	11	+	−	Acid	6	1
12	12	+	+	Acid	7	1
10	10	+	−	Acid	7	1
11	11	+	−	Acid	8	1
10	10	+	−	Acid	10	1
10	10	+	−	Water	10	7
11	11	+	−	Acid	12	1
11	11	+	−	Acid	12	3
12	12	+	+	Acid	12	1
13	13	+	−	Water	12	1
13	13	+	−	Acid	13	2
13	13	+	+	Acid	14	2
11	11	+	−	Water	14	4
15	15	+	−	Water	15	15
13	13	+	+	Acid	16	1
14	14	+	+	Water	17	1
13	13	+	−	Acid	17	1
13	13	+	−	Water	17	1
13	13	+	−	Water	18	1
13	13	+	−	Acid	18	1
12	12	+	−	Water	19	1
11	11	+	−	Water	20	12
11	11	−	−	Acid	21	0
12	12	+	+	Water	22	1
14	14	+	−	Acid	23	1
11	11	−	−	Acid	23	0
14	14	+	−	Water	28	1
13	13	+	−	Water	28	5
10	10	+	+	Water	28	7

TABLE 7—*Concluded*

SIZE		SIGN OF LIGHT REACTION		MORE CO_2	MINUTES IN HCl	MINUTES BETWEEN REVERSAL AND TEST
+	−	HCl	Control			
10	10	−	−	Water	29	0
11	11	+	−	Acid	30	5
11	11	+	−	Water	31	6
13	13	+	−	Water	33	1
10	10	−	−	Water	33	0
12	12	+	−	Water	33	1
12	12	+	−	Water	35	1
12	12	+	−	Water	37	1
15	13	+	−	Water	37	1
13	13	+	−	Water	38	38
13	13	+	−	Water	41	1
13	13	+	−	Water	41	14
11	11	+	−	Water	42	33
13	13	+	−	Water	44	1
11	11	−	−	Acid	44	0
10	10	−	−	Acid	48	0
13	13	+	−	Water	51	14
13	13	+	−	Water	58	3
13	13	+	−	Water	59	7
11	11	+	−	Water	67	0
13	13	+	+	Water	70	30
11	10	+	−	Water	78	10

TABLE 8

Showing relative carbon dioxide production of experimental and control nymphs analyzed on the basis of length of exposure to hydrochloric acid. This table is based on table 7

MINUTES IN HCl	MORE CARBON DIOXIDE	
	HCl	Water
Part I. Showing all tests listed in table 7		
1– 5	6	1
6–10	8	2
11–15	5	3
16–20	2	5
21–25	3	1
26–30	1	4
31–40	0	7
41–50	2	4
51–60	0	3
61–70	0	2
71–80	0	1

TABLE 8—*Concluded*

MINUTES IN HCl	MORE CARBON DIOXIDE	
	HCl	Water
Part II. Showing results of the biometer tests made within two minutes after reversal		
1– 5	6	1
6–10	7	1
11–15	5	1
16–20	2	3
21–25	3	1
26–30	1	2
31–40	0	5
41–50	1	2
67	0	1

TABLE 9

Showing the effect on carbon dioxide production of treating positive nymphs with N/25 hydrochloric acid until they became negative

SIZE		SIGN OF LIGHT REACTION		MORE CO_2	MINUTES IN HCl	MINUTES BETWEEN REVERSAL AND TEST
HCl	Control	HCl	Control			
13	13	–	–	Water	2	2
12	12	–	–	Acid	3	3
13	13	–	–	Acid	3	2
13	13	–	–	Acid	3	3
10	10	–	+	Acid	3	2
12	12	–	–	Acid	4	1
13	13	–	–	Acid	4	4
14	14	–	+	Acid	9	1

TABLE 10

Showing the relative carbon dioxide production of negative nymphs made positive by treatment with N/25 hydrochloric acid, compared with those similarly treated but not reversed

SIZE		MORE CO_2	MINUTES IN HCl		MINUTES BETWEEN REVERSAL AND TEST
+	–		+	–	
13	13	+	2	2	1
14	14	+	4	4	2
12	12	+	3	3	1
14	14	+	10	10	1
12	12	+	13	13	13
14	13	+	14	14	3
12	12	+	15	15	3
12	12	+	15	15	6
11	11	+	25	25	2
11	11	Both same	32	29	3
11	11	+	9	21	2
13	13	+	15	15	2

EXPERIMENTS WITH POTASSIUM CYANIDE

In the experiments with the negative Leptophlebia potassium cyanide had proved an effective reagent in causing reversals. With these Heptageninae at a concentration of N/500 it was almost as effective in causing reversals as hydrochloric acid. Of thirty tests run, 57 per cent were reversed by the cyanide while the controls showed no reversals at all. Biometer tests proved that the cyanide clearly depressed the nymphs. The details are listed in table 11 and specimen results are shown in chart 3. In all cases the nymphs were washed in water after treating with KCN in order to keep the potassium from interfering with the CO_2 determination.

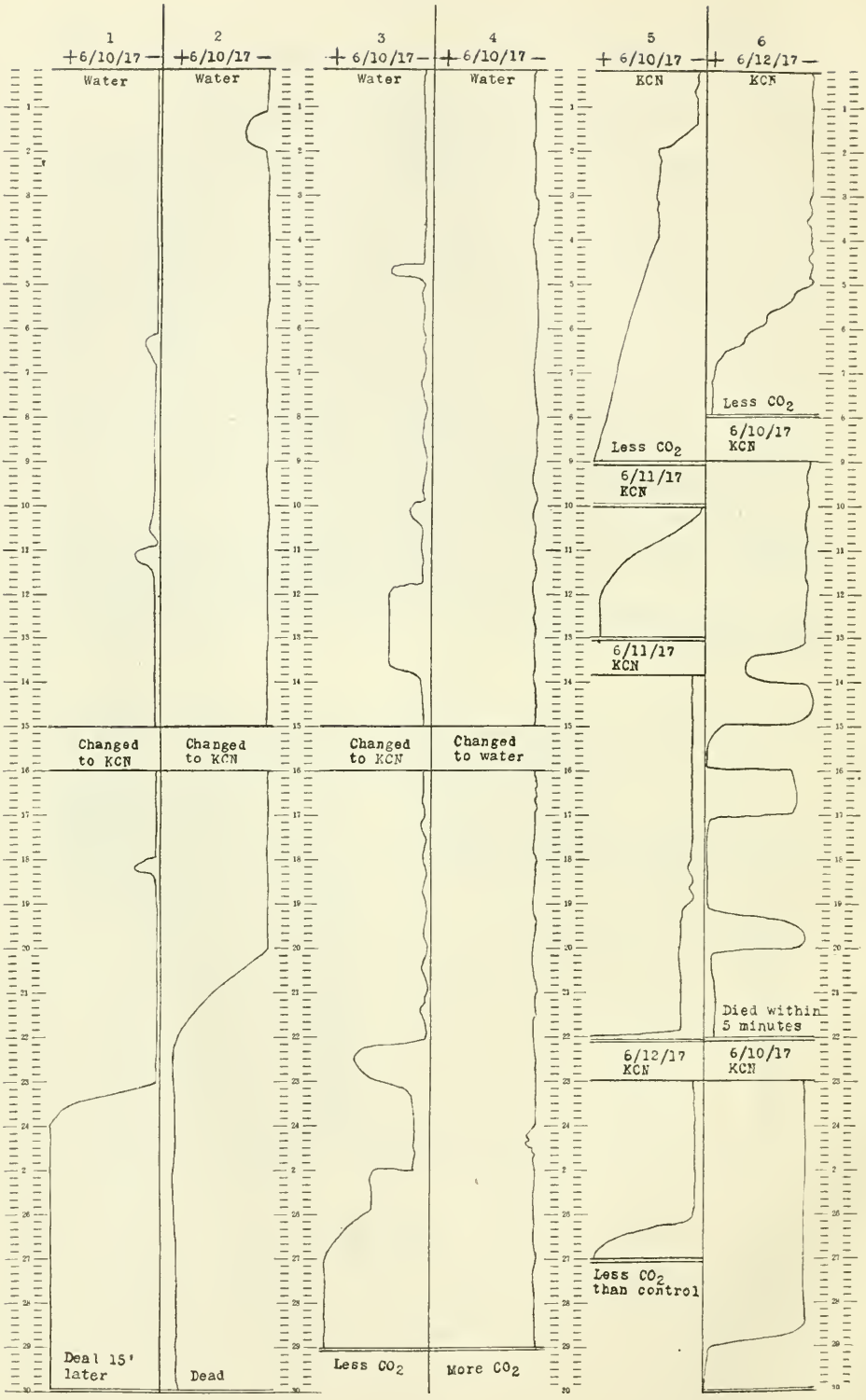
In addition to the carbon dioxide tests, one nymph died while moving positive, another just after reaching the positive end. In all ten nymphs died in the experimental dishes as a result of the cyanide treatment, of these six had reversed their reaction to light shortly before dying.

TABLE 11

Showing the effect of N/500 potassium cyanide upon nymphs negative to light and upon their rate of carbon dioxide production

SIZE		SIGN OF LIGHT REACTION		MORE CO_2	MINUTES IN KCN	MINUTES BETWEEN REVERSAL AND TEST
KCN	Water	KCN	Water			
13	13	+	−	Water	3	2
12	12	+	−	Water	4.5	1
14	14	+	−	Water	6	3
13	13	+	−	Water	7	2
12	12	+	−	Water	8	2
13	13	+	−	Water	6	1
13	13	+	−	Water	9	3
13	13	+	−	Water	14	4
13	13	+	−	Water	16	5
12	12	+	−	Water	19	2
12	12	+	−	Water	41	1

Chart 3


1
+6/10/17−
Water
Changed to KCN
Dead 15' later
2
+6/10/17−
Water
Changed to KCN
Dead
3
+ 6/10/17−
Water
Changed to KCN
Less CO_2
4
+ 6/10/17−
Water
Changed to water
More CO_2
5
+ 6/10/17 −
KCN
Less CO_2
6/11/17 KCN
6/11/17 KCN
6/12/17 KCN
Less CO_2 than control
6
+ 6/12/17 −
KCN
Less CO_2
6/10/17 KCN
Died within 5 minutes
6/10/17 KCN

EXPERIMENTS WITH ALCOHOL AND STRYCHNINE

Ethyl alcohol had less marked effect in causing reversals among these Heptageninae than with the species used in the first part of the work. In twenty-five well-studied cases, only five nymphs were by treatment with 2 per cent solution reversed. Of the fifty-two accompanying preliminary or control readings, one nymph became positive. When alcoholic nymphs were tested in the biometer (table 14), it was found that alcohol acts here according to its usual effect as a narcotic, first stimulating and later depressing.

Some typical results obtained with the alcohol treatment are given in chart 4. This shows two tracings of the reactions of nymphs that were not reversed by the treatment and of one that was, together with a type of control reaction which occurred frequently.

Some preliminary experiments with strychnine showed a marked increase in the activity of the nymphs without a reversal of their light response. The animals usually remained at the negative end of the dish in almost continual motion. In the biometer, they usually showed a markedly increased carbon dioxide production.

I am not able to state why these nymphs did not reverse their light reactions, but offer these results with alcohol and strychnine as evidence that all stimulation and depression do not cause reversals in phototaxis.

Chart 3 Showing the light reactions of ten nymphs treated with potassium cyanide together with three preliminary test periods and one complete control. In the cases where the preliminary and control graphs are not given it is understood that they are in no essential respect different from those shown. In order to economize space the time spent in changing from water to cyanide is not fully shown as it was in chart 2. Where such a change is indicated it must be understood that two or three minutes elapsed. In all cases given in columns 5 and 6 the control, with which the carbon dioxide production of the treated nymphs was compared, is not given in the chart. For text reference see p. 535.

Chart 4

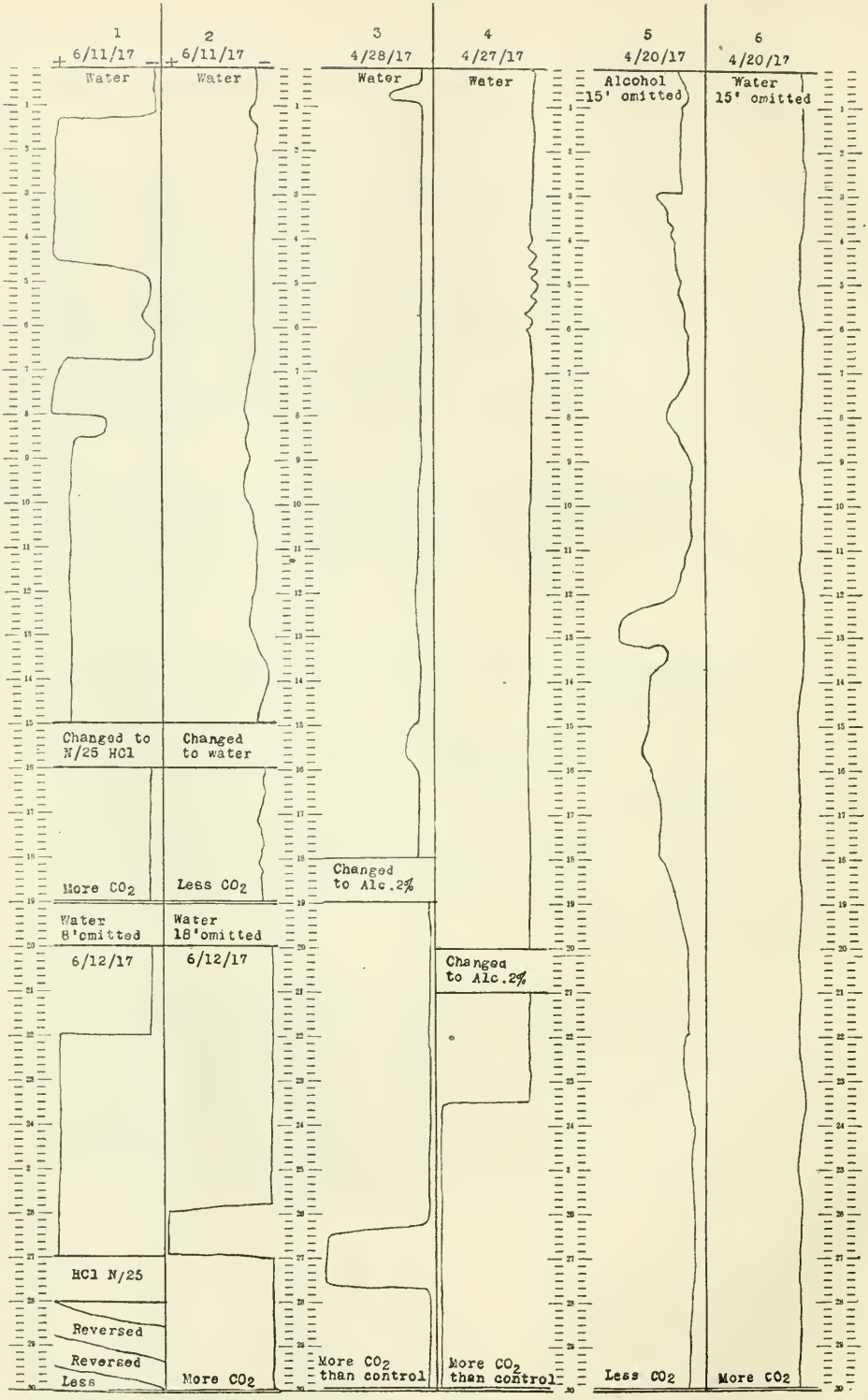

1
6/11/17
Water
Changed to N/25 HCl
More CO_2
Water 8'omitted
6/12/17
HCl N/25
Reversed
Reversed
Less
2
6/11/17
Water
Changed to water
Less CO_2
Water 18'omitted
6/12/17
More CO_2
3
4/28/17
Water
Changed to Alc.2%
More CO_2 than control
4
4/27/17
Water
Changed to Alc.2%
More CO_2 than control
5
4/20/17
Alcohol 15' omitted
Less CO_2
6
4/20/17
Water 15' omitted
More CO_2

TABLE 12

Showing the effect of 2 per cent ethyl alcohol upon the phototactic reaction of nymphs negative to light and upon their carbon dioxide production

SIZE		SIGN OF LIGHT REACTION		MORE CO_2	MINUTES IN ALCOHOL
Alcohol	Water	Alcohol	Water		
10.5	10.5	+	+	Alcohol	7
8.5	8.5	+	+	Alcohol	8
12.0	12.0	−	−	Alcohol	9
9.0	9.0	−	−	Alcohol	9
11.0	11.0	−	−	Water	9[1]
9.0	9.0	−	−	Water	9[1]
8.0	8.0	−	−	Alcohol	10
11.5	11.5	+	+	Alcohol	10
10.0	10.0	−	+	Water	10[1]
10.5	10.5	−	−	Water	10[1]
11.0	11.0	+	+	Alcohol	11
9.0	9.0	−	−	Alcohol	12
11.0	11.0	−	−	Alcohol	12
11.0	11.0	−	−	Alcohol	13
9.0	9.0	−	−	Alcohol	16
10.5	10.5	+	−	Alcohol	28
10.0	10.0	−	−	Water	44
11.5	11.5	−	−	Water	50
8.5	8.5	−	+	Water	50
10.5	10.5	−	−	Alcohol	52
11.0	11.0	−	−	Water	55
10.0	10.0	−	−	Water	83
9.0	9.0	−	−	Water	180

[1] These control nymphs were more active than the experimental ones before the biometer test was made.

Chart 4 Showing the reactions of two positive nymphs to light after treatment with N/25 hydrochloric acid together with their controls and the result of treating three nymphs with 2 per cent ethyl alcohol with one control tracing. The control shown in column 6 remained quietly at the negative end during almost all of its exposure and yet gave more carbon dioxide than the more active alcohol treated nymph whose response is shown in column 5. The nymph shown at the bottom of column 1 became negative immediately upon treatment with the acid. The experimental dish was then twice turned end for end each time the nymph moved directly away from the light.

DISCUSSION

Since this is an inquiry into the problem of a possible relationship between the sign of the phototactic reaction and the general rate of metabolism of May-fly nymphs, it is pertinent to question the effectiveness of our means of measuring the rate of the metabolic processes. The cyanide-resistance method is best checked by comparing results obtained with it with those given by Tashiro's method of determining carbon dioxide production. This has been done for the isopod Asellus communis (Allee and Tashiro, '14). There it was found that the two methods gave the same results in direct tests and that two isopods subjected to daily variations of oxygen tension for ten successive days with daily quantitative estimations of carbon dioxide production by Dr. Tashiro behaved according to expectation based upon previous experience with the cyanide method.

Regarding the work with Tashiro's biometer in measuring the carbon dioxide production, I consider this the best, though by no means the fastest, method of making such comparative carbon dioxide tests as those recorded in this paper. It is less complicated than the electrolytic determination of the hydrogen ion concentration and more accurate than the colorimetric methods. The instrument is somewhat complicated in appearance, but it is in reality as simple in manipulation as a modern microscope equipped with oil-immersion lens, mechanical stage, and camera lucida. I have repeatedly demonstrated end points to coworkers at Woods Hole and even to college freshmen. My only change from Tashiro's technique ('17, p. 109) consists in the use of a low-power binocular in reading end points.

The sources of error in the method as applied to May-fly nymphs are:

1. May-fly nymphs are water-dwelling animals and were tested in as nearly a dry atmosphere as possible.

2. Five minutes or more intervened between the time the nymphs were taken from the water and the first indication of the relative rate of carbon dioxide production. During this interval the nymphs must be picked up, partially dried, and placed in glass containers.

Regarding these points it must be borne in mind that the readings here given are all comparative ones in which the control and experimental nymphs were treated exactly alike. Since one must needs be taken from the water before the other, this was varied in the different experiments. The nymphs often lived twenty-four hours in the apparatus and at times they lived as long as forty-eight hours in the slight amount of moisture present.

3. Difference in carbon dioxide production may be due to difference in movement. This source of error was eliminated by the simple method of throwing out all tests where movement occurred.

4. Unconscious personal preference for one of the nymphs producing more carbon dioxide. This was eliminated by the experimenter's ignorance of which was experiment and which was control.

The relationship between the general metabolic processes of animals and their reaction to light may conceivably be one of the following:

1. Conditions that depress positive animals may make them negative (Mast, Bohn, Drzewina) and conditions that stimulate negative animals may make them positive (Holmes, Carpenter, Bohn, Jackson).

2. The above relationship may be reversed (Phipps in part).

3. Conditions that stimulate animals may cause reversals of their normal reaction or vice versa.

4. Conditions that markedly change the metabolic rate may cause reversal of either positive or negative animals.

5. Changes of metabolism accompanying changes in light reactions may be incidental or resultant rather than causal.

6. The relation between metabolic processes and the reaction to light may vary in different species so that no general law can be worked out.

7. There may be no relationship between the rate of metabolism and the phototactic reaction.

With the positive Epeorus nymphs only depressing agents were used and these caused reversals. With both the negative

species both stimulation and depression resulted in reversal and in positive members of the Lake Forest Heptageninae stimulation of positive nymphs caused reversal. In Leptophlebia thirty-six tests with hydrochloric acid showed no effect on the average resistance to potassium cyanide. The biometer tests with the Heptageninae give the explanation. When first subjected to the acid the nymphs are stimulated; later they are depressed. If taken during the first period the resistance to cyanide would offset that of the later period and so yield an average about the same as the control. An examination of the carbon dioxide production records shows that about as many nymphs were stimulated as were depressed by the treatment, so that a general average not considering the time factor would show reversals with no relation to the rate of carbon dioxide production.

In the May-fly nymphs studied the results obtained demonstrate that there is a relationship between the rate of metabolic processes and the sign of the phototactic reaction. It is also clear that reversals are accompanied by either a marked stimulation or marked depression. The experiments indicated but do not prove that the stimulation or depression is causal. From one point of view it makes little difference whether the metabolic changes are causal or only symptomatic; the fact that they are correlated at all is important.

If the change in metabolic conditions is causal, the fact is evident that all changes do not cause reversals. This was shown particularly by the action of the ethyl alcohol upon the Lake Forest nymphs. This stimulated and later epressed the nymphs with or without an accompanying reversal. On the assumption that metabolic change is causal the non-action of alcohol in 80 per cent of the cases might be explained by supposing that it did not cause a change quantitatively large enough. This suggestion is supported by the observation with the other species that alcohol accompanied by decrease in temperature ws more effective in producing reversals than alcohol alone and by the fact that in general these species were more susceptible.

The idea that a certain quantitative change must occur before reversal in light reaction takes place is also supported by carbon dioxide determinations made before the nymphs were exposed to light. In some of these, as, for example, the results shown in columns 5 and 6 of chart 1, the nymph with the higher speed of carbon dioxide production became positive upon exposure to light while the other was decidedly negative. It more frequently happened that both nymphs so treated were negative in spite of the fact that one had a higher rate of metabolism than the other. Evidently the biometer is more sensitive to changes in carbon dioxide production than is the light reaction.

Strychnine, again, did not cause a high degree of reversals, but it did strongly stimulate the nymphs. There is no question but that this stimulation is as strong as that caused by hydrochloric acid which caused a high percentage of reversals. This difference in result can only be explained by the assumption that while light reversals are accompanied by changes in metabolism that are probably causal, all such changes do not cause reversals in reaction to light.

The untreated positive nymphs were found to give off more carbon dioxide than untreated negative ones. These negative animals often moved back and forth in the dishes, spending the major part of the time at the negative end. Some nymphs do this more than others. This brings them to the positive end more frequently and gives them more opportunities to come to rest there. A reversal apparently on this plan is shown in column 4, chart 1. It appears that this is one mechanism for reversal to light and here the relation between metabolic rate and the reversal is obvious. The higher the metabolic rate, the greater the tendency to move back and forth; the more random movements, the greater the chance of becoming acclimated to the positive end and of reversing the normal reaction.

All reversals even under the influence of light alone were not of this type, witness column 3, chart 1. This reversal, like most of those experimentally obtained by the use of chemicals, was not preceded by random movements, but took place as though the animal had suddenly discovered the attractiveness of the positive end and must needs go there even though it died in the attempt

(as in cyanide reversals). This excursion was often the first one the nymph had made to the positive end and frequently the nymphs remained there until they died. The reason for the relation of metabolic rate with this type of reversal is not evident, especially when the reversal followed a very strong depression.

An explanation of reversals sometimes advanced (Ewald, '13) is that animals change in their sensitivity to light and hence reverse their reactions. On this basis the change in sensitivity is causal; the light reversal and change in carbon dioxide production, resultants. In May fly nymphs these supposed resultants are correlated in such a way that either stimulation or depression may accompany reversals from negative to positive light reactions. This means that either increasing or decreasing sensitivity to light will make negative May-fly nymphs positive, or that an increase (or decrease) in sensitivity wil cause now depression and now stimulation. Either of these necessary assumptions expand the sensitivity hypothesis beyond the limits justified by known facts.

CONCLUSIONS

The light reactions of the positive May-fly nymph, Epeorus, was reversed by treatment with alcohol, lowered temperature, calcium chloride, and other reagents. Nymphs so reversed had a lower rate of metabolism, as measured by resistance to potassium cyanide, than normal untreated nymphs.

The negative nymph, Leptophlebia, was similarly reversed with accompanying stimulation or depression as measured by resistance to the cyanide.

A negative nymph belonging to the Heptageninae was reversed in its light reactions with accompanying increase or decrease in carbon dioxide production as measured by Tashiro's biometer.

The experiments conclusively demonstrate that the phototactic reaction of these nymphs is correlated with the metabolic condition and indicate, but do not prove, that certain changes in metabolism cause the reversals in reaction to light.

All nymphs that reversed their light reactions were either stimulated or depressed, but stimulation or depression did not necessarily involve phototactic reversal.

LITERATURE CITED

ALLEE, W. C. 1914 Certain relations between rheotaxis and resistance to potassium cyanide in Isopoda. Jour. Exp. Zoöl., 16, pp. 397–412.

ALLEE, W. C., AND TASHIRO, SHIRO 1914 Some relations between rheotaxis and the rate of carbon dioxide production in Isopods. Jour. An. Beh., 4, pp. 202–214.

BOHN, GEORGES 1912 Les variations de la sensibilité en relation avec les variations de l'État Chimique Interne. C. R. Acad. Sci. Paris, 154, pp. 388–391.

CARPENTER, F. W. 1905 Reactions of the pomace fly Drosophila ampelophila Loew to light gravity and mechanical stimulation. Am. Nat., 39, pp. 157–171.

CHILD, C. M. 1913 Studies on the cynamics of morphogenesis and inheritance in experimental reproduction. V. The relation between resistance to depressing agents and rate of metabolism in Planaria dorotocephala and its value as a method of investigation. Jour. Exp. Zoöl., 14, pp. 153-206.

1913 a. Certain dynamic factors in experimental reproduction and their significance for problems of reproduction and development. Arch. Entw. Mech., 35, S. 598–641.

DRZEWINA, ANNA 1911 Sur la résistance des crustacés au cyanure et les effets sensibilisateurs de cette substance. C. R. Soc. Biol., 70, pp. 855–857.

DRZEWINA, ANNA, ET BOHN, GEORGES 1912 Modifications des réactions des animaux sous l'influence du cyanure de potassium. Ibid., 70, pp. 855–857.

EWALD, WOLFGANG F. On artificial modification of light reactions and the influence of electrolytes on phototaxis. Jour. Exp. Zoöl., 13, pp. 591–612.

GEPPERT, J. 1899 Über das Wesen der Blausäure-vergiftung. Zeitschr. klin. Med., 15, S. 208–307.

HOLMES, S. H. 1905 The reactions of Ranatra to light. Jour. Comp. Neur., 15, pp. 98–112.

HYMAN, L. H. 1916 On the action of certain substances on oxygen consumption. I. The action of potassium cyanide. Am. Jour. Physiol., 40, pp. 238–248.

JACKSON, H. H. T. 1910 Control of phototactic reactions in Hyalella by chemicals. Jour. Comp. Neur. and Psych., 20, pp. 259–263.

LOEB, J. 1904 The control of the heliotropic reactions in fresh-water crustaceans by chemicals. Univ. of Calif. Publ. in Physiol., 2, pp. 1–3.

LOEB, J. 1912 Comparative physiology of the brain and comparative psychology.

MACCURDY, HANSFORD 1913 Some effects of sunlight on the starfish. Sci., N. S., 38, pp. 98–100.

MAST, S. O. 1911 Light and the behavior of organisms. Wiley, 410 pp.

PHIPPS, C. F. 1915 An experimental study of the behavior of Amphipods with respect to light intensity, direction of rays and metabolism. Biol. Bull., 28, pp. 210–222.

Shelford, V. E. 1913 Animal communities in temperate America. Chicago, 362 pp.

Tashiro, Shiro 1913 A new method and apparatus for the estimation of exceedingly minute quantities of carbon dioxide. Amer. Jour. Physiol., 32, pp. 137-145.

1917 A chemical sign of life. Univ. of Chicago Press, 142 pp.

Wodsedalek, J. E. 1911 Phototactic reactions and their reversal in May-fly nymphs. Biol. Bull., 21, pp. 265–271.