THE REACTIONS OF CRAYFISHES TO GRADIENTS OF DISSOLVED CARBON DIOXIDE AND ACETIC AND HYDROCHLORIC ACIDS.[1]

EDWIN B. POWERS.

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I. Introduction.

The following experiments were undertaken to determine the reactions of crayfishes to gradients of acids, and, if possible, to determine the relation of the distribution of carbon dioxide contained in water to the natural distribution of the crayfishes. It was also hoped that something might be added to the present knowledge of the physiology of rapid modification of animals in

[1] From the Hull Zoological Laboratory, University of Chicago.

gradients. The experiments were conducted between January 13 and April 27, and between June 16 and July 31, 1913. The first set will be designated as low-temperature experiments and the second set as high-temperature experiments.

II. Material and Methods.

1. *Apparatus and Method of Experimentation.*

In the study of reactions of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids, the method and apparatus were devised by Shelford and Allee ('13) for the study of reactions of fishes to gases or solids in solution. The apparatus, a full description of which is given in Shelford and Allee ('13) "The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases" (pp. 225–229), consists of two tanks each 120 cm. long by 20.5 cm. wide by 14 cm. deep with outlets at the center of both sides, near the top. These outlets are guarded by a screen-bottomed tube which extends across the tank. The tanks are placed side by side in an aquarium, beneath a hood under identical and symmetrically surrounding conditions. Tap water was introduced at both ends through perforated tees behind screens. In the experiments with carbon dioxide, the flows were 600 c.c. per minute. At one end carbon dioxide was introduced into the inlet so that a gradient was produced lengthwise of the tank between the tap water and the water high in carbon dioxide. This was shown by titrations made of samples taken from different portions of the tank. In the acetic and hydrochloric acid experiments the conditions were the same, except for the flow at the tap water end and corresponding end of the control, which was 1,200 c.c. per minute, thus producing a sharper gradient. This flow was also used in all high temperature carbon dioxide experiments. The acetic and hydrochloric acids were introduced by means of a separate tee.

After everything was made ready for the keeping of records, the crayfishes were dropped into the center of the tank and were observed through a slit in the hood, and the back and forth movements recorded in the form of a graph on paper especially prepared for this purpose. Records were also kept of specific reactions observed in any individual. These records were made between the spaces set aside for the graphs. The previous history of the animals and the gradient used with a description of conditions of experimentation were entered at the top of the page. After the crayfishes were dropped into the middle of the tank they were disturbed as little as possible. At the end of each experiment a titration of samples of water taken from the two ends and center was made with sodium carbonate and recorded.

2. *Stock.*

The crayfishes were of the species *Cambarus propinquus* Gir., *C. virilis* Hag., *C. diogenes* Gir. and *C. immunis* Hag. They were all of medium size with the exception of a few specimens of *C. virilis* and *C. diogenes* which were above medium size. *C. propinquus* and *C. virilis* were all taken at New Lenox, Ill., from the pools just above and just below the rapids and from among the rocks of the rapids of Hickory Creek. The first stock was obtained December 25, 1912, and was kept in the laboratory in a large pan with the bottom covered with sand, gravel and some vegetation. The water was changed six times per week. When brought in a few died during the first three to five days, due possibly to the sudden change of temperature, but after this there was little mortality. Stock obtained November 15, 1912, and January 25, 1913, which were kept in an aquarium, suffered the same experience when transferred to the pan. Stock obtained March 22 and 29 and June 21, 1913, was kept in an aquarium in which was a strong flow of water to lower the temperature to more nearly that of experimentation. These suffered no great mortality. *C. immunis* was obtained from sloughs, mostly from the bottom, and a few from burrows, and *C. diogenes* from burrows only, along the banks of the same sloughs near Clark Junction, Ind., June 20 and 24 and July 9 and 26. These were all kept in large glass jars into which fresh water was flowing. There was no great mortality experienced by these stocks.

3. *Habitat.*

C. propinquus is essentially a quiet water stream form. It inhabits the pools with more or less muddy bottom (Williams, '01). They hide under rocks or rest quietly on the bottom.

Sometimes they lie concealed in short burrows along the banks (Harris, '03). *C. virilis* is found to be absent from the muddier and shallower portions of the streams but is sometimes taken in ponds with *C. immunis* (Harris, '01), but is more often found in running streams, among the rocks of the more rapid portions (Harris, '03). They do not burrow except when the ponds begin to dry or winter approaches. Garman ('89) took this species from wells, Wilson's cave and streams. *C. diogenes* is a burrowing species and often makes mud chimneys, often burrowing in damp ground some distances from the open water, which it seldom enters except during the breeding season (Pearse, '10). *C. immunis* is a mud-loving species and is found mostly in small pools, though it sometimes occurs in brooks and rivers (Pearse, *l. c.*) (Harris, '03).

4. *Senses.*

The fact that crayfishes are sensitive to chemicals has been shown by several authors. Putnam ('75) noted that *C. pellucidus*, the blind crayfish, will hunt food when it is thrown into the water. Holmes and Homuth ('10) found that the whole body is more or less sensitive to olfactory stimuli, and that the antennæ, mouth parts and tips of the chelipeds are sensitive in the order named. Nagel ('94) observed the chemical sense in *Astacus*. Wright ('84), by a study of the antennules of *C. propinquus*, found that five of the eighteen segments, *i. e.*, eleven to fifteen inclusive, bear eight of the so-called olfactory organs and the distal nine fewer. Bell ('06) found that the crayfishes with which he worked reacted positively to meat juice and negatively to lavender water, acids and salts, and concluded that they are sensitive over the entire body but more in the anterior appendages than in other parts. Chidester ('12) found when meat was thrown into the water that crayfishes would approach and seize fresh meat quicker than they would meat dried in the air.

III. The Sensing of Carbon Dioxide and Acetic and Hydrochloric Acids.

The crayfishes sensed the carbon dioxide and acetic and hydrochloric acids when passing into the high acid concentration end of the experiment tank. This was indicated by certain specific reactions which were made as the crayfishes entered the higher concentration. Such reactions were waving the antennæ, moving the appendages, backing and walking with the legs extended. The waving and moving of the appendages were especially noted in the reactions of *propinquus*, *virilis* and *diogenes*, in the carbon dioxide experiments in which the concentrations of carbon dioxide in the two ends were 40 to 60 and 80 to 100 c.c. per liter respectively. The animal would also crouch down in the corner of the tank. This tendency to wave the antennæ and move the appendages was present in *virilis* in the acetic acid also. The backing reaction was not so common in the carbon dioxide experiments but was marked in the hydrochloric and acetic acid experiments.

IV. The Effect of Carbon Dioxide and Acetic and Hydrochloric Acids.

1. *The Effect on Reflex of the Crayfishes.*

The crayfishes not only detected the presence of the acids but were intoxicated or anæsthetized by them in the low temperature experiments, possibly due to the less regular movements of the animal. This is shown in graphs (Chart I.) by the longer periods of time required to cross the tank in the experiment than that required in the control. The effect was greatest in the experiments with high concentrations. The first effect of the carbon dioxide was to interfere with the correlation of movements and to cause the animal to carry the body high with legs extended. Progressively locomotion became slower and slower until it ceased, but the appendages were still moved one after the other. The crayfish would finally fall upon its back and continue to move its appendages for a short time, after which it would remain motionless as if dead. It would recover rapidly if placed in fresh water and after a short time move about normally. The acetic acid produced this same effect upon *virilis* but *propinquus*, *diogenes* and *immunis* were either not intoxicated by the acetic and hydrochloric acids or acted rapidly enough to get out of the high concentration before being greatly affected, (see Charts I. and II.). The movements of *virilis* were always more or less irregular. One of the individuals in experiment 21 was completely anæsthetized but recovered in eight minutes when placed in fresh water. At first it remained motionless in the fresh water but later revived and seemingly became normal.

2. *Anæsthesia and Death.*

An experiment was performed to determine the relative resistance of the four species of crayfishes to high concentration of carbon dioxide. Though the experiment was terminated before the crayfishes had all been killed, through an accident to the apparatus, it had been carried sufficiently far to determine the susceptibility of the four species to the carbon dioxide solution. The apparatus consisted of large glass bottles (Wells, '13) through which water containing from 6.92 to 7 c.c. of oxygen per liter and varying amounts of carbon was flowing. The amount of the gases were determined by titrations of samples of water collected from the over-flow. The temperature was 21.5° to 23° C.

It was found that in all cases the smaller individuals of a species died first. This was probably due to the greater proportion of surface to mass in the smaller specimens than in the larger ones, rather than a difference of susceptibility of the smaller specimens to the carbon dioxide solution. These are the same general results obtained by Wells (Wells, *l. c.*) with fishes.

All remained active in 50 c.c. carbon dioxide per liter. *Virilis* was anæsthetized by solutions of 120 to 145 c.c. per liter. *Propinquus* was not overcome but showed that they were effected, while *diogenes* and *immunis* showed the effect to a less extent. *Propinquus* was not anæsthetized as early as *virilis* but the time of death approached that of *virilis*, the small specimens of *propinquus* having died before the large *virilis*, but a medium sized *propinquus* survived all the specimens of *virilis*. *Diogenes* and *immunis* were much less susceptible to the carbon dioxide than either *propinquus* or *virilis*. All specimens of both *propinquus* and *virilis* died before the medium-sized specimen of *diogenes*. The specimens of *diogenes* and *immunis* died in the following order: One medium-sized *diogenes*, one *immunis*, one *diogenes*, one *immunis*, two *diogenes* and two of *immunis*. There were one *immunis* and one large *diogenes* alive when the experiment terminated. All the specimens of *immunis* were smaller in size than the smallest specimen of *diogenes*. From this data it seems the four species are susceptible to high concentrations of carbon dioxide in the following order: *virilis*, *propinquus*, *diogenes* and *immunis*.

V. The Reaction and Modification in Gradients.

The reaction of crayfishes in gradients is shown in Tables I. and II. Table I. for carbon dioxide, II. for acetic and hydrochloric acids, are arranged in order of sharpness of the gradient, *i. e.*, the difference between the concentrations of acid at the two ends. The four species are grouped separately in each table. Reaction is shown by the time preference for one end or the other, by turnings and by crossings of the center. There is also tabulated the modification of behavior by turnings accompanied by backing. The backing reaction is not indicated in the ratings (Table III.), which is the numerical expression of avoidance of ends when turnings and time spent in the halves of the tank are considered. The ratio of the increased concentration of the acid of the high end over that of the low end, or Weber's ratio, is tabulated for comparison with the difference of concentration of the acid at the two ends.

The crayfishes, when passing into the high concentration end of the tank, gave certain definite avoiding reactions, when not too much affected by the presence of the acid. Of these the reactions recordable in graphs are (1) turning upon encountering gradient, either the first time or after one or more invasions and (2) reactions which cannot be recorded graphically are turning accompanied by backing and crawling on the screen, out of the water, or attempting to crawl on the sides of the tank. Recordable reactions were in the most cases rhythmic and represented a rapid modification of behavior. Reactions of the first class are grouped in one column of the table, but where backing accompanied turning separate mention is made of this fact. Of the reactions that could not be graphically recorded, that of crawling on the screen is least definite as an avoiding reaction since it was noted in the controls also, though less than in the experiments. This reaction is probably due to thigmotactic

Table I.

Showing reaction of crayfishes in a gradient of carbon dioxide. In all experiments and controls three animals were tested simultaneously except in the 20-minute experiments and controls in which case each of the three individuals were tested separately, 20 minutes each. H indicates high carbon dioxide and L low carbon dioxide. Corresponding ends of control tank are designated by the same letters. Typical experiments, only, are given in table. Totals and per cent. are totals for all experiments and controls of each species of crayfishes in each gradient. The experiments of each species are grouped and arranged in order of sharpness of gradient between the two ends of tank. For gradient see Table III.

Experiment No.	*Cambarus* Species.	Carbon Dioxide in c.c. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
2	*Propinquus*. .	3.5	20.	27.9	72.1	51.	49.	23	40	17	4	3	2	5	0	0	1	6.	60
26	"	3.6	23.	21.3	78.7	37.1	62.9	48	36	31	7	7	12	0	0	0	0	17.5	40
5	"	47.4	94.	5.6	94.4	70.6	29.4	7	41	12	1	1	5	4	0	0	0	3.75	60
	Totals.							157	237	124	57	32	40	20	3	3	1		
	Per cent.									71	29	44.5	55.5						
24	*Virilis*.	1.5	9.5	41.7	58.3			39		30	23			2	0			19.	40
27	"	2.9	46.5	11.1	88.9	24.1	75.9	40	18	40	21	14	20	1	0	0	0	17.5	40
	Totals.							108	85	78	54	27	24	4	0	0	0		
	Per cent.									59	41	53	47						
44	*Diogenes*.	2.3	18.9	33.3	66.7	35.8	64.2	18	21	7	5	5	3	0	0	0	0	20.5	20
28	"	56.5	179.4	26.	74.	58.8	41.2	20	32	18	11	4	3	8	2	0	1	17.5	20
	Totals.							58	85	69	45	39	29	15	6	0	1		
	Per cent.									60	40	57.3	42.7						
45	*Immunis*. . . .	2.3	9.4	33.7	66.3	48.3	51.7	52	38	13	5	7	8	0	0	0	0	21.	20
46	"	2.7	24.5	48.5	51.5	54.6	46.4	37	37	13	11	1	2	1	2	0	0	20.5	20
	Totals.							139	65	49	33	8	10	2	3	0	0		
	Per cent.									59.8	40.2	44.5	55.5						

response as well as an avoidance of the acids. The tendencies to crawl out of the water and upon the side of the tank are more clearly acid avoiding reactions. In the controls there were no attempts to crawl on the sides of the tank and few animals showed an inclination to crawl out of the water.

1. *Carbon Dioxide Gradient.*

(*a*) *C. propinquus.*—The crayfishes, upon invading the high carbon dioxide end, showed a tendency to turn before reaching the screen, or they would back a short distance and then turn and pass to the lower carbon dioxide end. Of all the individuals tried 55 per cent. turned back on the first encounter of the gradient and 14.8 per cent. of these turnings were accompanied by backings (Table I.). There was a greater number of turnings from the high concentration end than from the low, there being a total of 124 from the high end to 51 from the low. The fact that turning from the high end is an avoiding reaction is emphasized by 16 per cent. of the turnings being accompanied by backing. There were fewer crossings of the center in the experiments than in the controls, in the latter there being a tendency to travel the entire length of the tank. In some cases the reduction of crossings of the center represents the extent of anæsthetization of the animals.

In the experiments with low concentrations (low 3.5 and high 20 c.c. of carbon dioxide per liter) and low temperature the turnings were rhythmical (Chart I., Expt. 2). Long invasions of the high concentration end were followed by shorter invasions and periods of rest at the low concentration end. These were then followed by very short invasions and very long periods of almost complete rest in the low concentration end. Later there would be a second similar period of invasion and rest. Three such periods are shown in the graph. Thus there is a period of increased sensitiveness or a period of increased reaction to the same incoming sensation, after invasion of the high concentration end. In either case there is a rapid modification of behavior.

With higher concentrations (24–47 c.c. per L., low end and 58–94 c.c. per L. high end), if the crayfishes were not too greatly overcome, there was greater rapidity of reaction and a more

Table II.

Showing reaction of crayfishes in gradients of acetic and hydrochloric acids. For further explanation see Table I.

Experiment No.	*Cambarus* Species.	Acetic Acid in g. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
14	*Propinquus*. .	.24	1.39	3.5	96.5	54.9	45.1	14	22	15	3	11	13	7	0	0	0	4.25	60
13	" . .	2.41	3.66		100.	61.3	38.7	0	16	4	0	6	6	2	0	0	0	4.25	60
	Totals.							45	93	81	15	33	34	33	0	1	0		
	Per cent.									84.5	15.5	49	51						
21	*Virilis*.	1.21	1.67	41.	59.	45.3	54.7	11	13	17	7	11	5	9	1	1	0	6.25	40
30	"	.266	3.926	3.2	96.8	28.2	71.8	11	15	22	6	6	7	7	0	0	2	16.5	40
35	"	2.88	28.99	23.2	76.8	53.8	46.2	11	24	13	2	6	1	11	1	2	0	17.	20
	Totals.							101	139	113	46	51	40	52	7	3	5		
	Per cent.									71	29	36	44						
31	*Diogenes*.	.086	.312	10.	90.	63.9	36.1	21	27	15	2	11	5	3	0	1	1	16.5	20
33	"	.014	.354	41.1	58.9	92.	8.	26	10	19	10	18	2	5	3	4	0	16.5	20
	Totals.							93	117	51	29	43	24	14	10	5	2		
	Per cent.									64	36	64	36						
37	*Immunis*. . . .	.078	.429	29.4	70.6	53.8	46.2	21	45	24	8	11	9	7	2	0	0	18.	20
34	"	.429	1.437	16.7	83.3	54.1	45.9	6	3	5	5	1	3	2	0	0	0	16.5	20
	Totals.							33	52	34	15	14	16	10	3	0	1		
	Per cent.									67.4	32.6	46.7	53.3						

TABLE II.—*Continued.*

Experiment No.	*Cambarus* Species.	Hydrochloric Acid in g. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
39	*Propinquus*. .	.0061	.0157	9.4	90.4	60.1	39.9	29	30	27	6	1	2	4	0	0	2	20.5	20
47	" . .	.0275	.0932	4.7	95.3	49.6	50.4	28	63	39	2	0	1	3	0	0	0	21.	20
	Totals.							57	93	66	8	1	3	7	0	0	3		
	Per cent.									89.2	10.8	25	75						
41	*Virilis*.	.0075	.067	12.4	87.6	49.3	50.7	11	32	24	8	2	6	14	2	0	2	20.5	20
	Per cent.									75	25	25	75						
42	*Diogenes*.	.0023	.0078	30.5	69.5			39		7	5			2	0			21.	20
43	"	.0053	.0702	13.8	86.2	39.3	62.7	18	49	14	6	11	13	2	0	0	3	21.	20
	Totals.							57	49	21	11	11	13	4	0	0	3		
	Per cent.									66.7	33.3								
40	*Communis*. . .	.0014	.0304	31.5	68.5	49.3	50.7	23	42	18	7	10	8	2	1	0	1	20.5	20
	Per cent.									72	28	44.5	55.5						

Table III.

Showing the vigor of reaction or rating of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids. The rating is obtained by subtracting the percentages given for the time preference for the two ends and subtracting the percentage of turnings from the two ends and adding the two remainders (which are considered of different signs since turnings are from opposite end to end preferred) and dividing by two. The table also shows difference in concentration between the two ends and the ratio of increase of gradient at high end over low end or Weber's ratio.

Experiment No.	*Cambarus* Species.	Grams per L. of Acid or c.c. per L. CO_2. L	Grams per L. of Acid or c.c. per L. CO_2. H	Difference in g. or c.c. per L. between Two Ends of Tank.	Weber's Ratio.	Rating. Expt.	Rating. Cont.	
22	*Propinquus*	1.5	11.8	10.3	6.8	7.		
2	"	3.5	20.	16.5	4.7	53.	9.	
26	"	3.6	23.	19.4	5.4	60.	26.	
8	"	120.	149.	29.	.24	32.	59.	
3	"	24.	58.	34.	1.4	40.	23.	Carbon dioxide gradient.
5	"	47.4	94.	46.6	.98	86.0	53.	
4	"	43.	90.	47.	1.09	+5.5	20.	
6	"	32.	82.	50.	1.5	32.	59.	
29	"	37.1	154.7	117.6	4.3	42.	32.	
24	*Virilis*....	1.5	9.5	8.	6.3	24.		Ratio of increase of gradient at high end over low end or Weber's ratio.
10	"	18.	54.	36.	2.	+43.	46.	
11	"	62.5	101.	38.5	.26	2.	54.	
27	"	2.9	46.5	43.6	15.	54.	17.	
9	"	86.	172.	86.	1.	38.	3.	
44	*Diogenes*...	2.3	18.9	16.6	7.6	24.	27.	
25	"	3.3	35.9	32.6	9.9	13.	1.5	
28	"	56.5	179.4	122.9	2.1	36.	1.	
45	*Immunis*..	2.3	9.4	7.1	3.	38.	2.	
23	"	1.3	9.6	8.3	6.4	+ 4.		
46	"	2.7	24.5	21.8	7.7	4.	21.	
17	*Propinquus*	.045	.183	.138	3.2	79.	25.	
16	"	.045	.3	.255	5.7	83.	9.	
15	"	.24	.69	.45	1.9	65.	7.	
14	"	.24	1.39	1.15	5.2	79.	8.	
13	"	2.41	3.66	1.25	.48	100.	11.	
18	*Virilis*....	.018	.114	.096	5.1	53.	11.6	
19	"	.018	.22	.202	11.3	52.	25.	Acetic acid gradient.
21	"	1.21	1.67	.46	.3	39.	8.6	
20	"	.122	.8	.67	5.5	15.	10.	
30	"	.266	3.926	3.66	13.7	75.	21.	
35	"	2.88	28.99	26.11	9.	63.	16.	
31	*Diogenes*...	.086	.312	.226	18.5	78.	1.4	
33	"	.014	.354	.3406	23.8	24.	92.	
38	"	1.027	2.938	1.911	18.	.6	2.2	
36	"	4.97	23.33	18.36	3.6	8.	4.7	
32	*Immunis*..	.0109	.1105	.0996	9.1	38.	20.	
37	"	.078	.429	.351	4.5	45.	1.2	
34	"	.429	1.437	1.008	2.3	33.	29.	
39	*Propinquus*	.0061	.0157	.0096	1.6	72.	26.	
47	"	.0275	.0932	.0657	2.3	90.	45.	
41	*Virilis*....	.0075	.067	.0595	7.9	63.	12.	
42	*Diogenes*...	.0023	.0078	.0055	2.4	28.		Hydrochloric acid gradient.
43	"	.0053	.0702	.0649	12.2	56.	7.	
40	*Immunis*..	.0014	.0304	.029	20.2	40.	6.	

rapid modification of behavior (Chart I., Expt. 5). Periodicity is still present but is marked by shorter invasions of the high end. This periodicity is better shown by individuals than by groups. The graph of experiment 5, Chart I., shows that there was a complete cessation of invasions of the high end after 48 minutes and the crayfishes were still resting in the low end after 80 minutes. Numerically expressed, 70 per cent. of all individuals tried showed modification.

(*b*) *C. virilis.*—*Virilis* oriented less definitely to the carbon dioxide gradient than *propinquus.* (Compare ratings, Table III.) The lack of orientation is shown by the percentage of turnings from the two ends and the time preference for one end or the other. This staggering is possibly due to the somewhat more concentrated solutions used, but may be explained as has been suggested by Shelford and Allee ('13) for swift water fishes. That is swift water fishes which encounter very little carbon dioxide may react less definitely to it than fishes which live more often in the presence of carbon dioxide. *Virilis* showed a tendency to crawl out of the water.

(*c*) *C. diogenes.*—*Diogenes* reacted less intensely to both the lower and higher concentrations of carbon dioxide in gradients than either *propinquus* or *virilis.* In experiment 44 with 18.9 c.c. carbon dioxide per liter at the high end there was no backing accompanying the turnings, while in experiment 28, with 179.4 c.c. carbon dioxide per liter at the high end, there were 44 per cent. of the turnings accompanied by backing and one of the turnings on first encounter was accompanied by backing. *Diogenes* also showed a marked tendency in the high concentration carbon dioxide gradient to move forward and then stop after which it would move forward again.

(*d*) *C. immunis.*—All experiments with *immunis* were with concentrations not running above 25 c.c. carbon dioxide per liter at the high concentration end. The avoiding reactions with the exception of experiment 45 were very low. There was a slight positive reaction in experiment 23 as is shown in Table III. There were two turnings from the high concentration end accompanied by backing in experiments 23 and 46 while at the same time there were 3 from the low end.

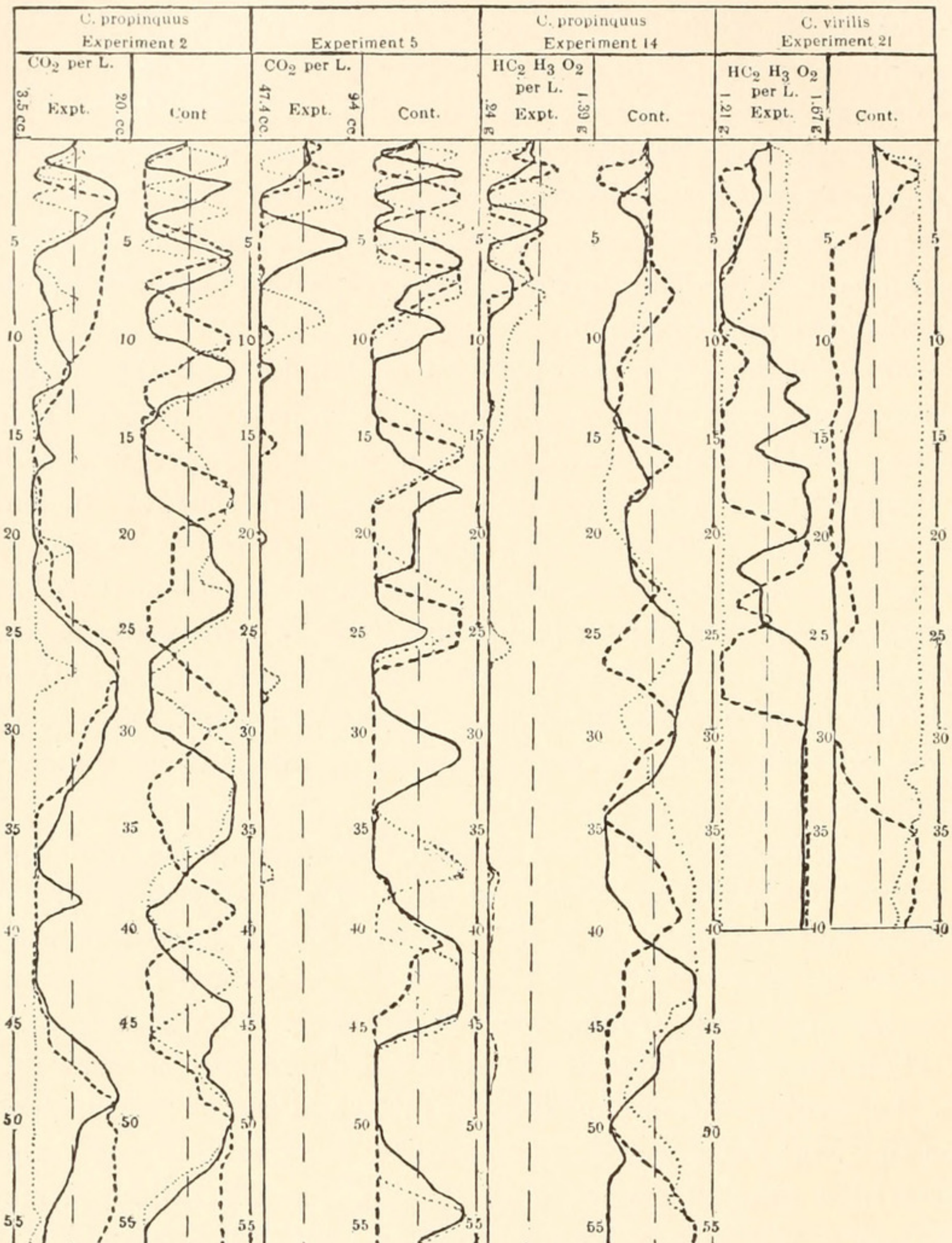

CHART I. Showing reaction of the crayfishes of gradients to carbon dioxide and acetic acid. The horizontal distance between the scales represents the length of the tank. The vertical scale represents time in minutes. Different tracings represent individual animals. Three animals were tested simultaneously.

2. *Acetic Acid Gradient.*

(*a*) *C. propinquus.*—The avoidance of the acetic acid in gradient was sharp and definite (see Chart I., Expt. 14). Orientation was also definite. There seemed to be an acceleration of all the reactions shown in the carbon dioxide experiments. Out of fifteen trials fourteen (93.3 per cent.) showed turning at first encounter of the high concentration, and of the fourteen, four started toward the high end without first having invaded the low or even a portion of it; 50 per cent. of the turnings were accompanied by backing. Only two individuals reached the screen in the acetic acid end. Of these two one crossed after handling; the other occurred in the lowest concentration of acetic acid used. There was, after a certain period of time, a cessation of invasions of the high acid concentration end; this period varied inversely as the total concentration of the acid. Experiment 14 varies slightly from this rule. There was also periodicity of invasions of the high concentration end (see Chart I., Expt. 14) as described in the carbon dioxide experiments.

(*b*) *C. virilis.*—These experiments can be divided into three groups, a low concentration, a high and a very high, *i. e.*, experiments 18 and 19 with a concentration of .0185 g. of acetic acid per liter at the low end and .1145 to .228 g. per liter at the high end; experiments 20 and 21 with .122 to 1.21 and .8 to 1.67 g.; and experiment 35 with 2.88 and 28.99 g. per liter of acetic acid in the low and high ends respectively.

In the low concentration experiments at low temperature there was a more definite orientation and a greater time preference for the low end. This is shown by the ratings (see Table III.). There was also a more or less periodicity of invasions of the high end with a complete cessation of invasions in experiment 19 after 45 minutes.

In the high concentration experiments at low temperature there was less orientation except in turning at the first invasion of the high end. There was but one individual (Expt. 20) that showed periodicity of invasions of the high end. The lack of orientation is shown by graph Expt. 21, Chart I. There was an increased tendency to crawl on the sides of the tank and out of the water. Thus there was a falling off of orientation and a substitution of crawling out of the water.

In experiment 30, in which the temperature was high and specimens above medium size were used, there was better orientation with a greater intensity of avoiding reactions as is shown by the rating and per cent. of time spent in the halves of the pan. See Tables II. and III., and Chart II., graph 30. While in experiment 35 where the concentration was very high there was a falling off of the intensity of the avoiding reaction due to one specimen becoming more or less anæsthetized. See Table II. and Chart II., Expt. 35.

(*c*) *C. diogenes.*—In the acetic acid experiments as well as the carbon dioxide experiments the intensity of the avoiding reactions are rather low with the exception of experiment 31 which is rather high, the rating being 78. There was a stronger tendency to stop and then move forward and with longer periods of rest than was noted in the carbon dioxide experiments.

(*d*) *C. immunis.*—The avoiding reactions of *immunis* to acetic acid was definite in the time spent in the halves of the pan, by turnings and by turnings accompanied by backings. See Table II., Chart II., Expt. 37 and 34. The intensity of the reactions in the acetic acid as well as in the carbon dioxide was rather low as is shown by Tables I. and II.

3. *Hydrochloric Acid Gradient.*

After having completed the experiments with carbon dioxide and acetic acid it was thought advisable to test the crayfishes with some inorganic acid and thus determine the difference or similarity of the reactions of the crayfishes to the different acids and to better compare the reactions of the four species of crayfishes. Hydrochloric acid was selected for this purpose. Since the ion constant of hydrochloric acid in very weak concentrations is approximately one (Stiegletz, '11) very low, concentrations were used in all the experiments. By an inspection of the tables it is seen that the same intensity of avoiding reaction was obtained in the low concentrations of hydrochloric acid but of high ion concentration as was obtained by the higher concentrations of acetic acid and carbon dioxide of lower ion concentration. Not only was the intensity of the avoiding reactions high in proportion to the concentration, but the reaction of all four species was more definite as is indicated by the turnings from the ends and turnings accompanied by backings. See Table II. and Chart II. Expt. 47, Chart II. was extended over a period

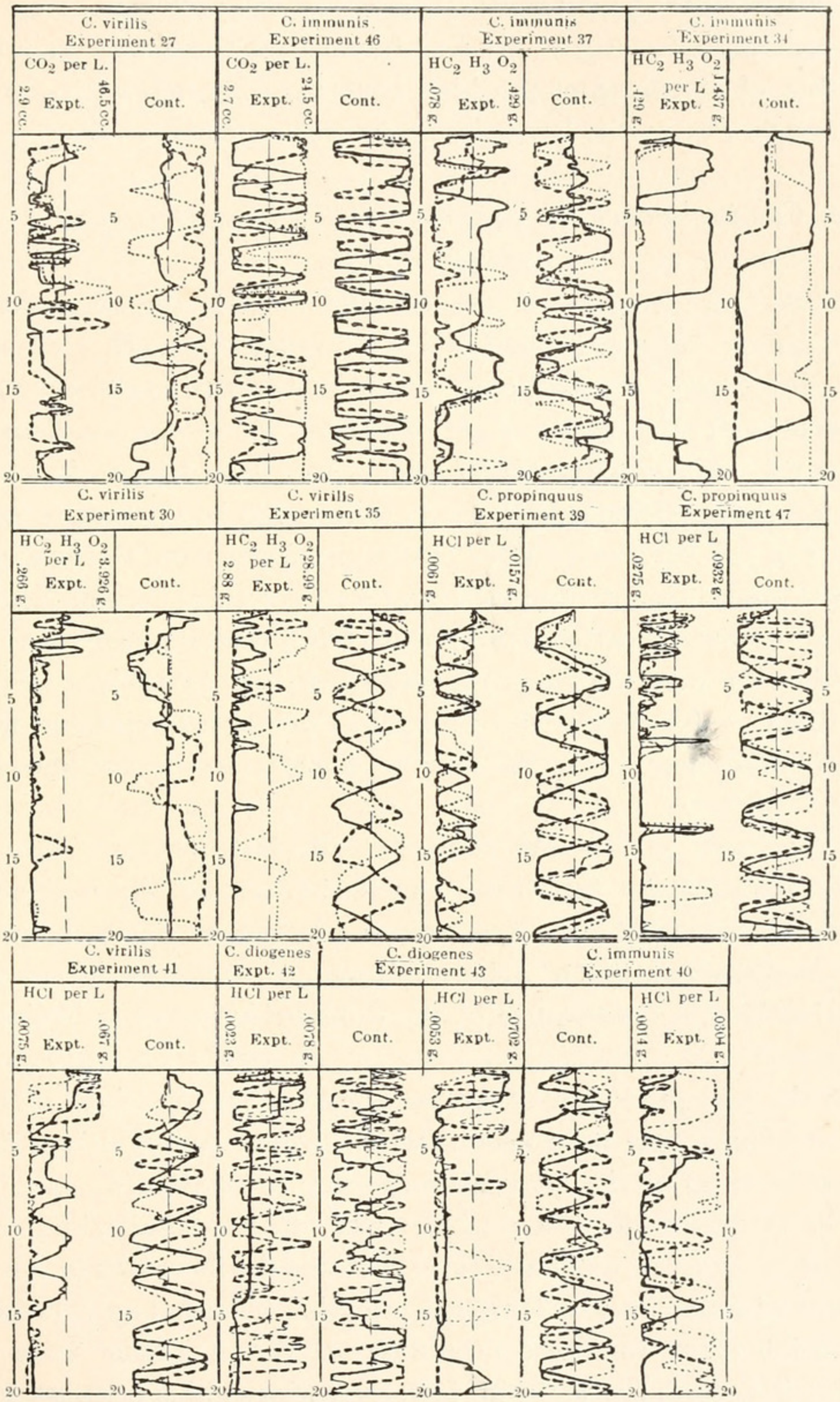

CHART II. Showing reaction of the crayfishes to gradients of carbon dioxide and acetic and hydrochloric acids. The horizontal distance represents the length of tank. The vertical distance represents time in minutes. Different tracings represent individual animals. In all experiments except experiments and controls 27 and 47 each animal was tested separately, 20 minutes each.

of forty minutes to determine the modification of behavior over a longer period of time. It was found that there was a similar rhythm of invasions of the high concentration end with a final coming to rest at the low acid concentration end.

4. *A Comparison of the Reactions of the Four Species of Crayfishes Tested.*

While there were noted specific differences in the reactions of the four species of crayfishes tested, all sensed the carbon dioxide and acetic and hydrochloric acids. Observations show that *propinquus* gives specific reactions and orients to a gradient of these substances, while *virilis* orients to a less degree; *immunis* to high concentrations, and *diogenes* to a still less degree to both high and low concentrations. Both *propinquus* and *virilis* which were tested at low temperatures were affected by carbon dioxide to the extent of intoxication, and *virilis* was affected more or less by the acetic acid. All species, so far as their avoiding reactions were noted, showed modifications of behavior and with *propinquus* there was a tendency to come to rest in the low end.

VI. General Discussion.

In reviewing the data it is seen that in each set of experiments all the types of behavior are increased in intensity with increase in the concentration of acid used, and the question as to the relation between the cause of the different types of behavior is suggested.

In the first place there must be a gradient before there can be orientation. This is in accord with Weber's law which states: "The increase of the stimulus necessary to produce an increase of the sensation bears a constant ratio to the total stimulus,"[1] *i. e.*, there must be a definite ratio between the increased intensity of the stimulus and the original stimulus before there can be a sensation of an increased stimulus. If the crayfishes were reacting in accordance with this law, the rating, which is the numerical expression of the degree of the reaction when both turnings and time preference are considered, should be in proportion to Weber's ratio, see Table III. By turning to the experiments

[1] James, The Principles of Psychology.

with *propinquus* in carbon dioxide and comparing the ratios of the increased concentrations at the high acid concentration end with the concentration at the low end of the low temperature experiments, it will be seen that there is no definite relation between the two. This comparison may be objected to on the grounds that *propinquus* was intoxicated by the carbon dioxide, but by turning to the acetic acid experiment, where *propinquus* was not affected there is seen the same variation between the ratings and Weber's ratio. At the same time it will be seen that there is a more definite relation between the rating and the total concentration of the acetic acid. In general the lowest total concentrations of acids have the lowest ratings. Experiments 15 and 16 are exceptions but still the range is not wide as compared with the great variation of Weber's ratios.

Now turning back to the carbon dioxide experiments (Table III., Expts. 2, 26 and 5) the intensities of the reactions are in reverse proportion to Weber's ratios, but are in direct proportion to the concentrations of the carbon dixoide solution used in the experiments. In the hydrochloric acid experiments (see Chart II. and Table III., Expt. 39 and 47) the intensity of the reactions conform with Weber's ratio, but at the same time it conforms with the concentrations of the hydrochloric acid used. Thus it is seen that the total concentration of the acid determines the intensity of the reaction. In other words the intensity of the reaction varies directly as the hydrogen ion concentration. This view is supported by comparing the ratings of the carbon dioxide and acetic and hydrochloric acid experiments. It is seen by comparing the carbon dioxide and acetic and hydrochloric acid experiments (see Table III.) that the rating on an average of the hydrochloric acid experiments are highest, acetic acid next and carbon dioxide lowest. This is due not to the higher molecular concentration of the hydrochloric acid over that of the acetic acid, since the molecular concentrations of the acetic acid were higher than that of the hydrochloric acid (Tables I. and II.), but to the higher ionization of hydrochloric acid over that of acetic acid and acetic acid over that of carbon dioxide in solution, thus giving higher ion concentrations.[1] These same

[1] Stieglitz's table of the ionization constants of acids, 1911.

points are suggested by the comparison of the ratings of each of the other three species in the hydrochloric acid, acetic acid and carbon dioxide experiments. It is interesting to note that when turnings only are considered that in all four species in the carbon dioxide, acetic acid and hydrochloric acid experiments that there is an increase of per cent. of turnings from the high acid concentration end over the low concentration acid end in the order named. Thus the hydrochloric acid again has the greatest intensity in avoidance of the high acid concentration end. These points are shown by the experiments with *propinquus* although the acetic acid experiments were of longer duration and were performed at a lower temperature than the hydrochloric acid experiments. The conclusion that intensity of negative reaction is directly proportional to the concentration of H ions can only be suggested, as sufficient data to warrant a definite conclusion are wanting.

The above suggestion might receive objection on the ground that there may be specific differences in the effect of the three acids used. Such an objection is supported by the fact that *propinquus*, in the low temperature experiment, is intoxicated by carbon dioxide and not by acetic acid. This apparent difference may, however, be explained on the ground that the carbon dioxide, since it diffuses more rapidly than the acetic acid, really produces a higher hydrogen ion concentration in the blood of the crayfishes than does the acetic acid, in spite of the fact that the latter acid is more highly ionized. The carbon dioxide would also tend to increase the carbon dioxide in the animal's blood by preventing the escape of the supply of this gas that is constantly being given off by the tissues of the animal. The acetic acid and hydrochloric acids would not offer any such hindrance to the diffusion of the internal gas into the water, and would not, therefore, be as detrimental as the carbon dioxide.

The periodicity and final cessation of invasion of the high concentration end of the experimental tank is a modification of behavior that may be brought about by increase in sensitiveness on the part of the crayfishes, or by a more rapid reaction to the same sensation. In the one case the cause is physiological, in the other the explanation must be psychological. If the modification is psychological then the animals must respond from associated memory. Shelford and Allee ('13, '14) have pointed out that it is hard to locate the things associated. Besides association formation is usually very slow for Yerkes ('08) states that 50 to 100 trials are necessary for the crayfish to form a perfect association in a simple labyrinth. The same slowness of modification would be expected of association due to a stimulus unless the sensitiveness of the animals was in some way progressively increased.

The modification is rapid, the number of invasions being sometimes but one before complete avoidance of the high end followed. This modification is probably due, as Shelford and Allee ('13) have pointed out, to increased sensitiveness on the part of the crayfishes and as they have further suggested, the greater sensitiveness may be the result of an increase in the hydrogen ion content of the blood of the animals.

In the cases where *propinquus* came to rest upon the screen in the low end and remained there for the rest of the experiment, the reaction may be considered the climax of the behavior modification, especially since the animals made this reaction more quickly in the presence of high total concentrations of acid than in the low. These points are shown by *propinquus*, Chart I., Expts. 5, 14. The explanation as to why the animals came to rest at all after being made more sensitive by the acid is not clear, but probably a combination of factors, one of which is thigmotaxis, were acting.

Shelford and Allee ('13) suggest carbon dioxide as a factor in determining the distribution of fishes and that the same may be true for crayfishes is suggested by the foregoing experiments. Crayfishes react to very weak concentrations of carbon dioxide and acetic acid and they were not overcome by the carbon dioxide except in concentrations higher than usually appear in natural waters. There seems also to be a correlation of the specific reactions of the two species with their respective habits. *Propinquus* is a pond form and its reactions were directive, while *virilis*, a rapid stream form, gave reactions which were much less directive. In natural waters carbon dioxide would be encountered in rather high concentrations by *propinquus* and that this species may react to these concentrations to its own advantage is indicated by the experiments heretofore described. *Virilis*, however, in its stream habitat would seldom encounter carbon dioxide concentrations of anything more than a very low degree and thus we find that this species does not react definitely to the gas. *Virilis*, then probably, while sensitive to carbon dioxide, orients itself in its environment by reactions to some other factor or factors. *Immunis* and *diogenes* are pond forms and are less sensitive to the acids but both avoid high concentrations. *Diogenes* although shown to be more sensitive than *immunis* orients less definitely as is shown by the per cent. of turnings from the high and low concentration acid ends. The specificities are again in coördination with the habitats of the species, *immunis* being a pond mud loving form, remaining in burrows only at times while *diogenes* remains almost wholly in burrows.

Summary.

1. Crayfishes sense the increase in carbon dioxide and acetic and hydrochloric acids in a gradient.

2. Both *propinquus* and *virilis* are intoxicated by carbon dioxide; *virilis* is also intoxicated by acetic acid but to a lesser degree.

3. The four species are susceptible to high concentrations of carbon dioxide and when subjected to high concentrations die in the following order, *virilis*, *propinquus*, *diogenes* and *immunis*.

4. *Propinquus* reacts negatively to the higher concentrations of carbon dioxide in a gradient, but when the total amount of acid present is large, the negative reaction may be interfered with by the direct detrimental effect of the acid.

5. *Virilis* reacts less definitely to the higher concentrations of carbon dioxide in a gradient than does *propinquus*. This is true whether the total concentration of the acid is large or small.

6. Both *diogenes* and *immunis* react more or less irregular to carbon dioxide due possibly to the lesser sensitiveness of these two species to this acid.

7. Both *propinquus* and *virilis* react negatively to the higher concentrations of acetic acid in gradients of this acid; *propinquus* reacts definitely in the presence of both high and low total concentrations; *virilis* reacts definitely to low total concentrations,

but not so definitely to high total concentrations; *diogenes* reacts irregular and less intense than the first two species while *immunis* reacts more definitely but with low intensity.

8. All four species react more strongly to hydrochloric acid than acetic acid and more strongly to acetic acid than carbon dioxide.

9. The intensity of avoiding reactions of all species to all acids tested as is shown by turnings only are in the following order; *propinquus, virilis, immunis* and *diogenes.*

10. The intensity of avoiding reactions of all four species varies directly as the total concentrations of the acids, and probably directly as the hydrogen ion concentration.

11. Rapid modification of behavior is shown by all four species. This modification may be due to the increased sensitiveness on the part of the animals, the increased sensitiveness being the result of higher ion concentration in the animal's blood.

12. The specific reactions of the crayfishes in gradients of carbon dioxide may be correlated with their habitats.

Acknowledgments.

I wish to express my appreciation and sincere thanks to Dr. V. E. Shelford, under whose direction this work was undertaken, for his constant help and many suggestions; also to Mr. M. M. Wells for assistance in determining the species of crayfishes used, and for suggestions during the performing of the experiments and writing of the manuscript.

Trinity University,
Waxahachie, Texas.

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