STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. IV. — LAWS OF CHEMOTAXIS IN PARAMECIUM.[1]

By H. S. JENNINGS.

[*Dartmouth College, Hanover, N. H.*]

1. Introduction.

IN the second paper of this series of Studies,[2] I have given an account of the mechanism of the reactions of Paramecium that requires a revision (for this animal) of the usual conceptions conveyed by the term chemotaxis. It was there shown that Paramecium reacts in essentially the same manner to all effective stimuli, and that the direction of motion in the reaction has no relation to the position of the source of stimulus, so that the animals are not directly attracted or repelled. When the animals are stimulated in any way, they swim backward, turn toward the aboral side, then swim forward over the new path so determined, without regard to the position of the stimulus. The terms positive and negative chemotaxis thus lose their content almost entirely, at least in the construction which is often put upon them. Nevertheless it will be convenient to employ these terms to express the fact that the Paramecia do, in the manner detailed in the paper above referred to, form collections in certain regions (positive taxis), and leave other regions empty (negative taxis). These phenomena retain their biological significance in full, whatever the means by which they are brought about. In view of the mechanism of the reactions, the following

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[1] Scientific Results of a Biological Survey of the Great Lakes, directed by Jacob Reighard (Ann Arbor, Mich.), under the auspices of the U. S. Commission of Fish and Fisheries, No. 2. Published by permission of the Hon. George M. Bowers, Fish Commissioner.

[2] The following papers have appeared in the series of Studies of which this is the fourth: I. *Reactions to Chemical, Osmotic, and Mechanical Stimuli in the Ciliate Infusoria*, Journal of physiology, 1897, xxi, pp. 258–321. II. *The Mechanism of the Motor Reactions of Paramecium*, This journal, May, 1899, ii, pp. 311–341. III. *Reactions to Localized Stimuli in Spirostomum and Stentor*, American naturalist, May, 1899, xxxiii, p. 372. These papers will be referred to in the following as I, II, and III, respectively.

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definitions may be given. Certain substances in solution set up in a Paramecium random movements, the direction of movement being changed at the time the stimulus begins to act and frequently so long as it continues to act. As a result of these undirected motions the Paramecium is in time, by the laws of chance, carried out of the region of the substance causing them, and prevented from re-entering. Hence toward these substances it may be said to be negatively chemotactic, and the substances may be called repellent. Other substances cause no motor reaction whatever, so that the Paramecia tend to come to rest within them, and these substances are further of such a nature that they throw the Paramecia into a peculiar physiological condition, such that coming in contact with a fluid not containing these substances causes the motor reaction above characterized, so that the animals are returned into the region of the substance causing no motor reaction. Hence if this region is small a dense assemblage of Paramecia may be formed within it. Substances of this character may therefore be said to be attractive, and the Paramecia are positively chemotactic toward them. The terms repellent and attractive, negative and positive chemotaxis, as used in this paper, are then to be understood to have significations in accordance with the above definitions.

2. Relation of Chemotaxis to Benefit or Injury.

The present paper deals with the nature of the substances thus causing or failing to cause the motor reactions, and with the laws of their action. The first question which will be taken up is in regard to the relation of chemotaxis to the benefit or injury to the organism, caused by the given substance. As shown in II, the effect of the motor reaction is on the whole to carry the organism out of the sphere of action of the substance causing the reaction and to prevent its returning. Is the production of this reaction due to the fact that the substance causing it is injurious to the Paramecia? This is a question of some interest in view of a common theoretical explanation of the origin of chemotactic movements. It is said that through natural selection such individuals as are repelled by injurious substances would survive and perpetuate their kind, while those not repelled by these substances would be destroyed; the converse being true as regards beneficial substances. We might then expect to find that the individuals which now survive have become accurately adjusted in these matters, being repelled by any substance

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which injures them. Any injury might be conceived to result in the " organic analogue of pain," causing thus motor reactions which take the animal away from the source of injury.

We know already from the first of these Studies that the substances which cause the motor reaction most markedly, and hence give most clearly the phenomena of negative chemotaxis, are chiefly alkalies, while substances in which the Paramecia collect, giving the motor reaction only when they attempt to pass *out* of them, are substances having a weak acid reaction. Thus the distinction between repellent and attractive substances is, to a certain extent at least, based upon purely chemical differences. But it was shown in the same paper that many neutral salts likewise cause the reaction, so that the acid or alkaline character of the solution is not the only determining factor; further, that when acid solutions reach a certain strength, they too cause the same motor reaction as do other substances; it is only weakly acid solutions that are attractive, while stronger acid solutions are repellent. It is a well-known fact that organisms are often thus negatively chemotactic toward substances to which in a weaker solution they are positively chemotactic.

Now, what is the basis of this change of reaction with a change in the strength of solution? The solution of course becomes more injurious as it becomes stronger, and the answer to this question which lies nearest is that the negative chemotaxis of the organisms is due to the injuriousness of the solution. May we say that as soon as an acid solution becomes equally injurious with the repellent alkaline solution, it too becomes repellent? And can we generalize the answer and say that the animals are throughout negatively chemotactic to certain substances in virtue of the fact that these substances are injurious to them?

If the injurious quality of the substance is the determining factor in causing the reactions which result in negative chemotaxis, then evidently two substances which are equally injurious must have equal powers of repelling Paramecia, and if one substance is injurious in a weaker solution than a second, then also must the first substance be repellent in a weaker solution than the second. In other words, the repelling powers of the two substances must be proportional to their injurious effects.

This gives a method of testing the question. We may determine the weakest solutions of two or more substances that repel the Paramecia, and note the ratio of their strength; then determine the

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weakest solutions of the same substances that will kill the Paramecia in a given time, noting their ratios as before. If the two ratios are approximately the same — that is, if the repellent powers are proportional to the injurious effects — then the evidence is in favor of the proposition that the production of the motor reaction resulting in repulsion depends upon the injurious effect of the substances; if the ratios are not the same, then the relation between repellent power and injuriousness is not one of direct dependence.

I have compared in this way a considerable number of substances. The repellent powers of different chemicals were tested in the manner described in I and II for studying chemotaxis, — by introducing with a pipette drawn to a capillary point a drop of the substance beneath the cover-glass of a slide of Paramecia. The substances to be tested were dissolved in proper proportions in distilled water. In distilled water alone the Paramecia form collections (as shown in I), so that the repellent powers of the solutions are due wholly to the dissolved substance. For finding approximately the weakest solution of a substance that causes repulsion, it is convenient to proceed as follows: A series of covered watch-glasses are filled with solutions of the given substance, each successive watch-glass with a solution one half or one fourth as strong as that in the preceding one. Thus we shall have perhaps the following solutions: $\frac{1}{5}$, $\frac{1}{20}$, $\frac{1}{80}$, $\frac{1}{320}$, and $\frac{1}{1280}$ per cent, etc. The Paramecia are placed on a slide beneath a long cover-glass supported at the ends by bits of glass tubing. Now with the capillary pipette drops of solutions of different strengths are introduced beneath the four corners of the cover-glass, — under one corner a drop of $\frac{1}{5}$, under the next $\frac{1}{20}$, then $\frac{1}{80}$, and $\frac{1}{320}$ per cent. We shall probably find that some of the drops remain empty, while others are quickly filled with Paramecia. Suppose that the $\frac{1}{5}$, $\frac{1}{20}$, and $\frac{1}{80}$ per cent remain empty, while the $\frac{1}{320}$ per cent is quickly filled. It is thereupon evident that the weakest solution which repels the Paramecia lies somewhere between $\frac{1}{80}$ and $\frac{1}{320}$ per cent. By making proper intermediate grades between these two, approximately the weakest solution that repels the Paramecia can usually be determined very expeditiously. All the precautions insisted upon in I as necessary for carrying on such experiments were of course rigorously observed.

I will present in detail the results in two typical cases, then summarize my results on the sixty-five substances tested, in the form of a table.

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For a first case we may take two chemically related substances, toward both of which the Paramecia show positive chemotaxis when the solutions are very weak. Chromic acid and potassium bichromate fulfil these conditions. These substances both have an acid reaction, due to the same component in each case, and the Paramecia gather in weak solutions of both of them. In stronger solutions both cause the motor reaction which results in repulsion. Is this due in each case to the injurious effect of the stronger solutions?

For potassium bichromate the weakest solution that repels the animals is about $\frac{1}{20}$ per cent. For chromic acid the weakest solution which sets the motor reaction in operation is about $\frac{1}{150}$ per cent. The ratio of repellent powers is therefore 7.5 to 1 in favor of the chromic acid.

The injurious effects of the solutions are now tested as follows: Watch-glasses with solutions of different strength are arranged in series, as above described, and into each a drop of water swarming with Paramecia is introduced and quickly mixed. The solutions are made in such a way that after the introduction of the water containing Paramecia they are of the recorded strength. The weakest solution in which all the Paramecia are killed in one minute is thus determined for the two substances.

For the potassium bichromate it is found in this way that the weakest solution which kills the Paramecia in one minute is a one per cent solution, — that is, a solution twenty times as strong as the weakest solution that repels them. In the chromic acid they die somewhat more quickly in a $\frac{1}{150}$ per cent solution, — a solution of the *same* strength as the weakest which causes repulsion. The ratio of the injurious effects of the two substances is therefore as 150 to 1 in favor of the chromic acid.

Comparing the two ratios, we find that they are strikingly unequal. While chromic acid has but $7\frac{1}{2}$ times the repellent power of potassium bichromate, it is 150 times as injurious. Potassium bichromate repels in a strength $\frac{1}{20}$ of that which is severely injurious; the chromic acid does not repel until it has reached a strength which is already destructive. In the latter case the repellent power is evidently due directly to the injurious effects; repellent power and injurious effects appear at the same point as we pass from weaker to stronger solutions. If the conduct of the Paramecia is observed under the microscope as they swim backward after coming in contact with a drop of $\frac{1}{150}$ per cent chromic acid, it is at once evident that they are

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decidedly injured, and frequently they die after swimming backward a little distance. The motor reaction is thus not set in operation by the chromic acid until the Paramecia are already injured and it is too late to save them. When such a drop is introduced beneath the cover-glass, the Paramecia are therefore killed by scores and the drop is soon surrounded by a ring of the dead animals. If a drop of a solution somewhat weaker than $\frac{1}{150}$ per cent is introduced, the Paramecia enter it directly, forming a dense gathering, and after a time it may be observed that many individuals of the group are dead or severely injured. In the case of the potassium bichromate, on the other hand, the Paramecia are never injured whatever the strength of solution used; they gather in a ring about a drop of strong solution, but do not enter it deeply enough to be injured.

For these two substances then the test results negatively; potassium bichromate has twenty times the repellent power it should have if repellent power depends alone on the injuriousness of the solutions.

We may next test two substances which do not attract the Paramecia in any strength, and which are not injurious in virtue of their chemical properties at all, but only as a result of their osmotic pressure. As such substances we may select sodium chloride and cane sugar. Repeated comparative tests with isotonic solutions of these two substances indicate that neither is immediately injurious to the Paramecia except through osmotic pressure; strong isotonic solutions of the two substances kill the Paramecia in approximately the same period of time. Thus if Paramecia are introduced at the same time into 1 per cent sodium chloride solution and 9.64 per cent cane sugar (which is isotonic with 1 per cent solution of sodium chloride), in both cases nearly all the individuals are found to be dead twenty minutes after they were introduced, and in neither solution does the proportion of dead markedly preponderate. This result for Paramecium differs from that gained by True[1] for Spirogyra; in the case of Spirogyra sodium chloride was found to have a distinct toxic effect in addition to its osmotic properties.

The weakest solution of sodium chloride which repels the Paramecia is from $\frac{1}{10}$ to $\frac{1}{20}$ per cent. The weakest solution of cane sugar which sets the motor reaction clearly in operation is 10 per cent. Taking the repellent solution of sodium chloride as $\frac{1}{10}$ per cent, the

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[1] TRUE: Botanical gazette, 1898, xxvi, p. 407.

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repellent power of sodium chloride is to that of cane sugar as 10 to $\frac{1}{10}$, or as 100 to 1. On the other hand, as stated above, 1 per cent sodium chloride has the same injurious effect as 10 per cent cane sugar, so that the injurious effects of sodium chloride are to those of cane sugar as 10 to 1. The repellent power of the cane sugar, like that of the chromic acid, is plainly due to its injurious properties. The motor reaction is not set in operation until plasmolysis has begun, as shown by the evident shrinkage of the bodies of the animals, and it is usually too late at this time to save them from destruction. If a drop of strong sugar solution is introduced into a slide of Paramecia, it soon becomes filled with the dead Protozoa. On the other hand a drop of sodium chloride is not destructive whatever its strength, since the motor reaction is set in operation long before the Paramecia have entered deeply enough to be injured. Sodium chloride has, as shown by the ratios given above, ten times the repellent power that it should have if repellent power depends entirely upon injurious effects.

A large number of compounds, chiefly inorganic, were tested as to the relation between repellent power and injurious effects, and it was found that the cases given above are typical for all. We can divide chemical substances as regards this matter into two classes, as shown in the following table. In the first column may be placed together substances in which, as in potassium bichromate and sodium chloride, the repellent power is great in proportion to the injurious effects. These substances all set the motor reaction in operation long before any injury has taken place, so that the Paramecia are not harmed in the least when a drop of one of them is introduced into a slide preparation of the infusoria. In the other column may be placed substances in which, as in chromic acid and cane sugar, the repellent power is slight in relation to the injurious effects ; the motor reaction in all these substances is set in operation only when the Paramecia are already injured and it is too late to save them. A drop of any one of these substances introduced beneath the cover-glass of a slide preparation of Paramecia proves exceedingly destructive.

We may for convenience classify the substances in the table also into those which are attractive to the animals in weak solutions (A), and those which are repellent in all effective solutions (B).

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TABLE I.

1. Repellent power strong in proportion to injurious effects: chemotaxis protective.	2. Repellent power very weak in proportion to injurious effects: chemotaxis not protective.
(A. *Attractive Substances.*)	
Sodium fluoride, Potassium bichromate, Ammonium bichromate, Potassium ferricyanide. (KBr), (KCl), (RuCl), (CsCl).[1]	HF, HCl, HBr, HI, H_2SO_4, HNO_3, Acetic Acid, Tannic Acid, Picric Acid, Chromic Acid, Potassium fluoride, Potassium permanganate, Ammonia alum, Ammonio-ferric alum, Chrome alum, Potash alum, $CuSO_4$, $CuCl_2$, $Cu(C_2H_3O_2)_2$, $ZnCl_2$, $HgCl_2$, $AlCl_3$.
(B. *Repellent Substances.*)	
LiCl, NaCl, (KCl), (RuCl), (CsCl),[1] LiBr, NaBr, (KBr), RuBr, LiI, NaI, KI, RuI, Li_2CO_3, Na_2CO_3, K_2CO_3, $LiNO_3$, $NaNO_3$, KNO_3, NaOH, KOH; $KBrO_3$, NH_4F, NH_4Cl, NH_4Br, NH_4I, $CaCl_2$, $SrCl_2$, $BaCl_2$, $Ca(NO_3)_2$, $Sr(NO_3)_2$, $Ba(NO_3)_2$.	Cane sugar, Lactose, Maltose, Dextrose, Mannite, Glycerin, Urea.

From this table and the foregoing discussion it is evident that the question proposed is to be answered in the negative. The injuriousness of a substance is not the determining factor in its repellent power, since the repellent power of different substances is not at all proportional to the injuriousness of their solutions. In the substances of the second column, such power as they have to set in operation the motor reaction of Paramecium is indeed evidently due directly to the injuries caused, though this power is nothing like so precise and strong as in the substances of column 1, not being sufficient even to prevent the Paramecia from entering and being destroyed.

The fact that the injuriousness of a solution is not the chief determining factor in its repellent power seems to indicate that the common theory, according to which the reactions of organisms have been fixed by natural selection through the destruction of the individuals not repelled by injurious substances, does not contain the whole truth.

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[1] In the case of the substances enclosed in parentheses, the reactions of Paramecia from different regions varied, as described in a subsequent part of this paper.

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Probably many of the substances experimented with have never been met by the Paramecia in the course of their evolution, and their reaction toward these must be determined by other laws. Possibly their reactions are adjusted by natural selection to some small number of substances, while to other substances they react by analogy as it were, — by the similarity of their effects to those of the substances to which the reaction has been adjusted by natural selection. Thus it may be that repulsion for some alkali plays an important part in the biology of the animals, and that the reaction toward other substances of this sort is due to their similarity to the primary one. We know that to the tendency to collect in weak solutions of carbon dioxide is due the apparently gregarious habit of the Paramecia, and this probably serves a useful purpose somewhere in the life of the animals. It may be that the tendency to gather in other acid solutions, even such destructive ones as sulphuric acid and copper sulphate, is a sort of reflection of this tendency to collect in carbonic acid, and that natural selection has had no opportunity to correct this tendency directed toward injurious substances. That there is much opportunity for natural selection to act is clear when one tests any chemical substance by bringing a drop of it among a large number of Paramecia. There is always a number of individuals who do not give the typical reaction, some being much more and some much less sensitive than the majority.

Another consideration of importance is the following: It may not be at all the *direct* effects of a substance on the Paramecia, through which natural selection has adjusted the reaction toward that substance. Thus it may be that in solutions of a certain nature, harmless in themselves, the Bacteria which form the food of the Paramecia are never found; natural selection would then act by starving such Paramecia as were not repelled by these solutions.

3. RELATION OF CHEMOTAXIS TO CHEMICAL COMPOSITION.

If the injuriousness of substances is not the determining factor in their repellent power, what *is* the determining factor? An examination of the table above given brings out the following facts. All the substances in which the repellent power is relatively great contain a large proportion of some metal of either the alkali group (Li, Na, K, Ru, Cs, or NH_4) or of the earth alkali group (Ca, Sr, Ba). This is true of all, whether having an acid, a neutral, or an alkaline reaction. On the other hand most of the substances in the second column con-

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tain none of these elements. It is true that certain of these compounds, as potassium fluoride, potassium permanganate, and the alums do contain members of the alkali group. But in potassium fluoride the qualities of the compound are due almost entirely to the fluorine component, as is shown by the fact that this salt, like hydrofluoric acid itself, attacks glass energetically. In potassium permanganate and the alums the proportion of alkali metal is small, as is shown by their formulas. Thus the formula for potash alum is $KAl(SO_4)_2, + 12\ H_2O$, so that in the molecule composed of forty-eight atoms but one of these is K; the properties of these compounds are very little due to the alkali component. The proportion of alkali metal is very much larger in the substances of the first column. This dependence upon the presence and proportion of an alkali metal is in practice so striking that in working with salts whose action on the Paramecia is not yet known, it is usually possible to predict with certainty from the chemical formula into which column they will, upon experiment, be found to fall.

The results on these sixty-five compounds appear then to justify the following conclusion: Compounds which contain a large proportion of one of the alkali or earth alkali metals set in operation the motor reaction of Paramecium even when exceedingly weak; their repellent power is therefore great in proportion to their injurious effects, and the infusoria are not endangered by the presence on the slide where they are swimming of a drop of a solution of these compounds. Substances which do not contain a large proportion of one of these metals as a rule set in operation the motor reaction of Paramecium only when the animals are already injured; their repellent powers are very weak in proportion to their injurious effects, and a drop of one of them introduced into a preparation of Paramecia is very destructive to the latter.

The fact, however, that the injurious properties of a substance on reaching a certain intensity of action do produce the motor reaction is worthy of special notice; in all the substances in the second column the reaction is manifestly due directly to the injuries inflicted, without regard to the nature of the substance. Paramecium is not sufficiently sensitive to injuries to make the reaction thus produced of any protective value, at least under experimental conditions. But it may easily be conceived that this sensitiveness might be so increased that the reaction would be of the greatest importance for preserving the animals; it is probable that in many organisms this condition is real-

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ized. We seem to have in Paramecium the very lowest step in the production of reactions due to the "organic analogue of pain" (to use a phrase employed by J. Mark Baldwin), — a step so low that the power of thus reacting seems to have no value for the life of the organisms.

The chief determining factor in the repellent power of solutions is then, for Paramecium, of a chemical nature, with little relation to the comparative injuriousness of the solutions.

The conclusion above drawn as to the repellent power of the alkali metals is reinforced and extended by certain results gained in a study of the compounds of these metals with the halogens, — compounds of lithium, sodium, potassium, rubidium, and cæsium on the one hand, with fluorine, chlorine, bromine, and iodine on the other. These form a definitely circumscribed group of compounds, the characteristics of which, as is well known, vary in many respects with the molecular weights of the elements composing them. A qualitative and quantitative study of the reactions of the Paramecia toward these compounds was begun at Put-in-Bay Island, Lake Erie, during the summer of 1898, in the hope that such a study would throw light on the essential nature of chemotaxis. The work was not finished at Put-in-Bay, and on taking it up again at Hanover, N. H., it was found that the Paramecia here gave in part different reactions from those studied at Put-in-Bay. The differences are of such a nature as to be not unintelligible, yet it will be necessary to consider separately the results obtained at the two places.

The elements belonging to the group of halogens may be arranged in a series, beginning with that having the least atomic weight and proceeding to that which has the greatest atomic weight, as follows: fluorine (at. wt. 19), chlorine (at. wt. 35), bromine (at. wt. 80) iodine (at. wt. 127). As is well known, this is also the order of the chemical activity of these elements, the lightest being most active. In the same way the alkali metals may be arranged in a series beginning with the lightest, as follows: lithium (at. wt. 7), sodium (at. wt. 23), potassium (at. wt. 39), rubidium (at. wt. 85), and cæsium (at. wt. 133). This series also gives in a general way the order of chemical activity of the elements, the lighter ones being more active.

The compounds of these two sets of elements may be arranged in a table in such a way as to give the halogen series from above downward, the alkali series from left to right. In the ensuing table,

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arranged in this manner, are placed only the compounds which I was able to procure and use for studying the reactions of the Paramecia at Put-in-Bay. For convenience the hydroxides of the metals and the acids of the halogens are brought into the table, — the former constituting the lower row, the latter the right-hand column.

TABLE II.

	Li(7)	Na(23)	K(39)	Ru(85)	Cs(132)	
F(19)	....	$\underline{NaF}$	$\underline{KF}$	....	....	$\underline{HF}$
Cl(35) . . .	LiCl	NaCl	$\underline{KCl}$	$\underline{RuCl}$	$\underline{CsCl}$	$\underline{HCl}$
Br(80) . . .	LiBr	NaBr	$\underline{KBr}$	....	....	$\underline{HBr}$
I(127) . . .	LiI	LiI	KI	....	....	$\underline{HI}$
		NaOH	KOH			

In this table, as will be observed, the compounds of the lighter (and more active) metals are placed to the left; the compounds of the lighter (and more active) halogens in the upper row. In the right-hand upper part of the table are given therefore compounds of light, active halogens with heavy, inactive metals; in these the influence of the halogens might be expected to predominate. In the left-hand lower corner are given compounds of light, active metals with heavy, inactive halogens; in these the metal component might be expected to predominate.

Now some of these substances repel the Paramecia in any effective concentration; that is, they produce the motor reaction which results in carrying the Paramecia outside of their influence; these we may call the repellent substances. Others when weak do not cause the motor reaction when the Paramecia enter them, but when the animals attempt to swim *out* of the drop into the surrounding water the motor reaction occurs, and the Paramecia return into the drop. They thus form collections either within such drops or surrounding them in a close ring, — in the manner described in I, p. 269. These we may call attractive substances.

All the attractive compounds in the above table are underscored; those not underscored are repellent. It will be seen, first, that all the

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acids or hydrogen compounds of the halogens are attractive, the hydroxides, or hydroxyl compounds of the metals repellent. Further, that all the compounds of the lightest, most active halogen (fluorine) are attractive. Of the next lightest, chlorine, three salts are attractive and two repellent. Of the heaviest, most inactive halogen, iodine, all the salts studied are repellent. Of the lightest, most active metal, lithium, all the salts tested are repellent, and the number of repellent salts decreases as we pass toward the heavier metals. Thus the compounds of light, active metals with heavy, inactive halogens are repellent; those of light, active halogens with heavy, inactive metals are attractive. A diagonal line can therefore be drawn, as shown in the table, from the upper left hand to the lower right hand, separating all the attractive substances on the right from all the purely repellent substances on the left.

It thus appears that the Paramecia react to these compounds with relation to the elements of which they are composed. The metal components tend to repel the Paramecia; the halogen components to attract them. The acids of the halogens, in which the halogen may be presumed to give character to the compound almost alone, do not repel except in such solutions as to be immediately destructive, as previously discussed. The hydroxides, or bases, in which the metals give the character to the compound, are repellent in any solution strong enough to produce any effect whatsoever. Those compounds which are partly metal and partly halogen are attractive or repellent in accordance with whichever one is the predominant element.

The same conclusion is reached by a study of the exact method of behavior of the Paramecia toward the substances above classed as attractive. Toward these compounds the infusoria are as a rule *both attracted and repelled*. As just stated the acids are purely attractive, the bases purely repellent. Toward potassium fluoride, in which the halogen component is so active as to attack glass even in this salt, the animals react as toward a pure acid. Toward sodium fluoride, in which the fluorine is so bound by the sodium that the two produce a comparatively inactive salt, the Paramecia are both strongly attracted and strongly repelled. When a drop of one per cent sodium fluoride solution, for example, is introduced into a slide of Paramecia, it will soon be found that the animals have gathered in a dense ring about the drop, leaving its centre entirely free. In this ring they swim back and forth, giving the motor reaction whenever they come

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to the inner side of the ring, where the stronger sodium fluoride is, or when they come to the outer side of the ring, where pure water is. Sodium fluoride thus gives a striking example of apparently positive and negative chemotaxis to the same substance. Of compounds outside the group at present under consideration, potassium and ammonium bichromate and potassium ferricyanide show precisely the same phenomena.

The other salts classified in the table as attractive show the same phenomena as sodium fluoride, except that the attraction is less strong and the repulsion stronger. The Paramecia gather in a narrow ring about them, and this ring contains at a given time comparatively few individuals, though enough to make the ring evident as a rather sharply defined white line around the drop. If the individuals constituting this ring are closely observed, it becomes evident that the motor reaction is set strongly in operation when they come to the inner boundary of the ring, where the stronger solution of the salt is found, and much less strongly when they come to the outer boundary, against the ordinary water. Thus many individuals remain in the ring but a short time, finally crossing the outer boundary and escaping, so that the ring does not increase continually in numbers and size, as it does in the case of sodium fluoride; it consists of changing individuals, each one being detained usually but a short time. In some of the salts the ring thus formed is almost evanescent: for a short time it will be distinct, then almost disappear, then reappear again. Finally, about the salts which are not underscored in the table no ring of Paramecia is ever formed.

This formation of a ring of greater or less extent about solutions of various salts is a very curious phenomenon. The Paramecia are evidently thrown into such a condition by a very weak solution of the substance that the contact with the surrounding water now acts as a shock or stimulus to set the motor reaction in operation, — while this motor reaction is also induced by a stronger solution of the same salt, such as is found nearer the centre of the drop. In view of the facts adduced, it seems apparent that the shock on reaching again the outer water is due to the physiological effect of the halogen component, as this shock alone is produced in compounds in which, as in acids and potassium fluoride, the halogen is the element that chiefly gives character to the solution. On the other hand, the stimulus due to entering the salt itself is a result of the metal component, since this stimulus alone is produced in those compounds in which, as in

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hydroxides (and carbonates) the characteristics of the compound are due chiefly to the metal.

The above results were obtained at Put-in-Bay in a series of about 1000 tests of the substances in the table. The compounds used were tested many times, dissolved both in distilled water and in the same water in which the Paramecia were found, with every possible precaution to secure accuracy, and with uniform results as given above. But on continuing the series of experiments at Hanover, N. H., with the intention of filling the gaps in the table as above given, different results were obtained with the Paramecia of this region for certain of the salts. Toward KCl, KBr, RuCl, and CsCl the Paramecia of Hanover show only repulsion, without any sign of the formation of a ring about them, which was so evident at Put-in-Bay. Toward the NaF and KF on the other hand they react exactly as did those at Put-in-Bay, forming a ring about the NaF and reacting toward the KF as toward an acid. Toward all other members of the above table the Hanover Paramecia likewise react exactly as did those at Put-in-Bay. This variation is evidently not of a character to modify in any way the conclusions to be drawn. The Paramecia at Hanover simply fail to show a very faint reaction given by those from Lake Erie in the presence of certain substances; they seem therefore merely slightly less sensitive; in other respects the reactions are similar in all the Paramecia studied. The Paramecia at Put-in-Bay were cultivated in lake water containing decaying water plants, chiefly Nuphar, Naias, and Ceratophyllum. At Hanover the Paramecia came from cultures of hay, in water from small ponds of the neighborhood. Possibly the difference in the culture medium has something to do with the difference of reaction in the Paramecia of the two regions.

The reactions toward certain other substances may be stated briefly. Toward the carbonates of lithium, sodium, and potassium the reaction is the same as toward the hydroxides of these metals. Also toward the nitrates of these metals and toward the chlorides and nitrates of calcium, strontium, and barium the reaction is of the nature of repulsion alone, as toward the iodides, sodium chloride, etc., given in the table. Toward salts of the heavy metals, as cupric chloride, cupric sulphate, mercuric chloride, zinc chloride, aluminium chloride, the Paramecia are attracted, as toward acids.

From the facts given in the above table and discussion the following conclusions may be deduced: —

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1. The base-forming elements of the alkali and earth alkali groups when present in a compound tend to produce the motor reaction in Paramecia whenever the animals come in contact with these compounds, even in very dilute solutions. This may be put in terms of the modern theory as follows: the kations of the alkali and earth alkali groups have a strong tendency to induce the motor reaction in Paramecia. As a consequence these infusoria are kept from entering such solutions even when the solutions are very weak.

2. The ions of the acid-forming elements, such as the halogens, and in fact the anions of *all* acids, do not themselves tend to cause the motor reaction in Paramecia, except when present in such strength as to be immediately destructive. On the contrary, they tend to throw the Paramecia into such a physiological condition that contact with any solution containing no anions produces the motor reaction. As a consequence, Paramecia tend to congregate in regions where it is mainly the anions that give character to the solution.

The fact that the same result as for acids is obtained for salts in which the anion is much the most powerful component, as in potassium fluoride, shows of course that the results with acids are not due to the hydrogen ion of the acids.

3. Compounds that contain both kations of the alkali elements, and anions, may give a combination of these results, — a tendency to cause the motor reaction (with its reversal of cilia) both when the animals enter the solution (due to contact with the kations), and when they leave the solution (effect of the anions). In this case the Paramecia collect in a ring about a drop of the compound.

4. Salts that contain the relatively inactive kations of one of the heavy metals, as aluminium, copper, zinc, mercury, produce an effect due only to the anion, hence their effect is like that of an acid.

These conclusions in regard to the different effects of the anions and kations on the motor reactions of Paramecium certainly seem to invite a discussion of the relation of the activities of the ions during the passage of an electric current through a solution to the movements of the Paramecia under the same conditions. As is well known, when a continued current is passed through the water containing Paramecia, they all swim with one accord to the kathode, — thus in the same direction as that in which the metallic ions are moving. It is difficult to resist the impression that there must be some such relation between the movements of these ions and the movements of

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the organisms as has been suggested by Loeb and Budgett.[1] Yet a discussion of this matter will hardly lead to valuable results until it can be accompanied by a careful re-examination of the phenomena of electrotaxis, with especial reference to the relation of the electrotactic movements to the general motor reaction of Paramecium described in the second of these Studies. We possess already a detailed account of the movements of the cilia in electrotaxis, due to Ludloff,[2] but these observations, as well as my own observations on electrotaxis, published in I, were made before the general plan of the motor reactions of Paramecium was known, so that a re-examination might lead to the discovery of facts of fundamental significance.

4. Relative Repellent Power of Related Substances.

In the first part of this paper I have shown that with relation to their action upon Paramecium, chemical substances are divisible into two classes, in one of which the repellent power is very great in proportion to the injurious effects, while in the other class such slight repellent power as exists is due directly to injuries caused. In this latter class a quantitative study of the relative repellent powers of the different members shows of course that the power of setting in operation the motor reaction of Paramecium is in a general way proportional to the injuriousness of the solutions. But to what factor are the relative repellent powers among themselves in the members of the *first* group due? I have already shown that the relative repellent power of members of the first group, as compared with that of members of the second group, is due to the presence in preponderating amounts of some metal of the alkali or earth alkali series. But this leaves untouched the question of the relative repellent powers of members of the first group among themselves. For example, will sodium chloride be more or less repellent than potassium chloride, and why?

On the question of the relative repellent powers of different members of this group much labor was spent, with rather unsatisfactory results. This is due to the fact that the different compounds have specifically different ways of affecting the Paramecia, some modifying the general motor reaction in one way, some in another, — although never changing the essential character of the reaction. This is a matter worthy of special study. Chemotaxis is a complex pheno-

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[1] Loeb and Budgett: Archiv f. d. ges. Physiol., 1897, lxv, p. 518.

[2] Ludloff: *Ibid.*, 1895, lix, p. 525.

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menon, the motion to or from a substance being the indirect resultant of the specific physiological effects of this substance upon the different phases of the motor reaction. These specific physiological effects are therefore the primary phenomena. This fact was not recognized at the time my work was done, so that full records were not made on these points. On a few substances observations were made, which I will give here as examples. As shown in II, the typical motor reaction is as follows: (1) the cilia are reversed, causing the Paramecia to swim backward; (2) the aboral cilia of the anterior half of the body strike transversely toward the oral side, turning the animal toward the aboral side; (3) the cilia all strike backward as at first, carrying the animal forward over a path which lies at an angle to the original path. This reaction is given in characteristic form when the animal comes in contact with a weak alkali; all the parts are fairly though not excessively pronounced. On coming in contact with a strong acid solution, the Paramecia dart furiously backward a long distance. Then they turn and dart furiously forward, so that they often thus rush directly into the drop of acid, and perish. If their course after turning, however, tends to carry them away from the acid, when they strike the water into which no acid has diffused they react again by reversing and turning, — showing that the acid has put them into a peculiar physiological condition. Some acid substances cause the reaction with certain of its typical features nearly or quite suppressed. Thus when the animals swimming forward come in contact with diffusing mercuric chloride, they dart straight backward, the anterior end being curved a little to one side; at the same time the body begins to swell. Then they start straight forward again, the turning usually being nearly or quite omitted. Paramecia which come in contact with a drop of $\frac{2}{5}$ per cent calcium chloride solution while swimming forward, pass in without hesitation, but are excited and the forward course is hurried, so that they soon pass out. They thus never remain quiet in the calcium chloride, and as a result it remains nearly empty, the Paramecia gradually settling down elsewhere. Yet one cannot say that the calcium chloride repels the animals; it merely excites them, without causing even a change in the direction of motion. Paramecia introduced directly into 2 per cent barium nitrate solution are paralyzed instantly. But in three or four minutes they revive, and swim slowly about. In 2 per cent calcium nitrate solution, on the other hand, they are at once violently excited, swimming forward and backward swiftly. In solution of

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strontium nitrate they are likewise excited, but much less so. In 1 per cent sodium bromide and sodium iodide they first dart backward, then turn, then swim forward, then backward again, — repeating the entire typical motor reaction many times. By 1 per cent potassium iodide each part of the motor reaction is almost indefinitely prolonged; they swim backward perhaps ten minutes at a time, then they turn toward the aboral side, and *keep on turning*, whirling thus on the short axis for twenty minutes or more. In 10 per cent solution of cane sugar they at first give no reaction; gradually as plasmolysis occurs they begin to swim backward, then turn, then swim forward, repeating this, as in sodium bromide solution. It will readily be seen in view of these peculiarities in reaction, that it is not always possible to classify the conduct of the Paramecia at once into the two categories of attraction and repulsion.

Moreover, toward many compounds there exist great individual differences among the Paramecia as to the reaction given; at times a few individuals will swim directly into a solution, while the rest leave it empty. There is thus no absolute criterion for determining at what strength the repulsion of a solution really ceases. Moreover, the sensitiveness of the Paramecia to solutions of different strength of a given substance seems to vary according to something like Weber's law, — so that the least observable differences in reaction are between that due to a solution of given strength, and that due to a solution of twice this strength. It is often difficult to determine, for example, whether the repellent power of a substance ceases at $\frac{1}{40}$ or $\frac{1}{80}$ per cent; but upon this decision may depend our conclusion as to which of two substances shows the greater repellent power.

The following results upon certain salts are, however, based upon a very large number of experiments, and so far as they go, may be given with confidence. The method of experiment was that already described, the two substances being compared always under exactly similar conditions in all respects, by introducing a drop of each beneath the cover-glass of the same slide of Paramecia. A special study was made in this way of the halogen salts of the alkali metals, — the chlorides, bromides, and iodides of lithium, sodium, potassium, rubidium, and ammonium, together with the chloride of cæsium. This study was made at Hanover, and therefore upon Paramecia that were repelled by all these salts, so that the repulsion was not confused with any accompanying attraction.

As explained in connection with certain similar results given in I,

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it is not possible to give a figure which shall represent in a general way the weakest concentration of any one of these substances that causes repulsion in the Paramecia, on account of the very great variation in the reaction of Paramecia from different cultures and under different conditions. For example, on testing Paramecia from two different cultures at the same time, it was found that those from one culture were decidedly sensitive to lithium chloride in a $\frac{1}{40}$ per cent solution, leaving a drop of this strength empty for a long time, while those from the other culture were quite indifferent to the same chemical even in a strength sixteen times as great, swimming in and out of a drop of $\frac{2}{5}$ per cent solution of lithium chloride without reaction. But the *relative* sensitiveness of Paramecia to two or more different chemicals seems to remain fairly constant, so that the order of sensitiveness to different chemicals can be determined. I give therefore the order of sensitiveness to the different metal compounds of each halogen, — the test of sensitiveness being the relative strength of solution of the different substances that causes repulsion.

For the chlorides the order is as follows, beginning with the most repellent: LiCl, KCl, NaCl, RuCl, CsCl. That is, the repellent power decreases as the atomic weight of the metal component increases, except that the sodium compound is moved down one place, being less repellent than the compound of the heavier potassium. The weakest repellent solution varies from about $\frac{1}{40}$ per cent in the lithium chloride to about $\frac{1}{2}$ per cent in the chloride of cæsium.

For the bromides the order is, beginning with the most repellent, LiBr, KBr, NaBr, RuBr; for the iodides LiI, KI, NaI, RuI. In these two sets of salts the order is thus the same as for the chlorides. In general, therefore, the repellent powers of the halogen salts of the alkali metals varies inversely with the atomic weight of the metal components, except that in each case the sodium compound is less repellent than the heavier potassium compound. The ammonium salts were found in each case to possess almost exactly the same repellent power as the corresponding salts of potassium.

Neglecting for the moment the inverted order in the potassium and sodium salts, two causes suggest themselves for the inverse proportion between the repellent power and atomic weights of the elements. The halogen component remaining the same throughout a given series, the molecular weights of the salts of course increase with the atomic weights of the metal components. Hence the less repellent compounds have the greater molecular weight, so that a

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solution of given strength by weight of one of these salts contains fewer molecules than does the corresponding solution of one of the lighter salts. Supposing the molecules to have throughout the same repellent power, the solution of the lighter salt, containing more molecules, will of course be more repellent. A second cause may be the usual greater chemical activity of lighter elements. If both these causes are efficient factors, they of course reinforce each other in the series above given. The reason for the anomalous position of the sodium salts, as weaker than the potassium salts, does not appear.

On comparing the series of the different halogen salts of a given metal, as NaCl, NaBr, NaI, I have found it impossible, in spite of much labor directed upon this point, to determine satisfactorily the relative repellent powers. Apparently the three salts have almost exactly equal repellent powers, and many sets of experiments upon the corresponding series for each of the four metals, lithium, sodium, potassium, and rubidium, have not made it possible to differentiate them. In every case the repulsion was very nearly equal for all, and the slight differences observed in one set of experiments did not hold for the next. It is evident that the two factors given above as possibly reinforcing each other in causing the greater repellent power in the lighter salts of the metal series of a single halogen, would, in the halogen series of a single metal, work in opposite directions. The greater number of molecules in the lighter compounds (as of chlorine) would tend to produce greater repulsion. But the greater chemical activity of the lighter chlorine component would tend to neutralize the effect of the metal component, to which alone the repulsion is due. Possibly it is to this interference that the approximately equal repellent power of the chlorides, bromides, and iodides of a given alkali metal is due.

Much time was spent on a similar quantitative study of the nitrates of the alkali metals and of the earth alkali metals, but without clearly defined results. The different nitrates have specifically different effects on the Paramecia; the repellent power is the resultant of several factors, any one of which may vary independently of the others. Thus the phenomena differ in the different salts, and there is no certain criterion for comparing the repellent powers of two salts having different specific physiological effects.

The above somewhat fragmentary and partially negative results of quantitative studies are given, not only because the data obtained are of themselves of some value, but also because it is of importance to

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show which lines of investigation promise to be fruitful and which do not. This method of study has thrown chiefly a negative light upon chemotaxis, — showing what it is *not*. The evidence that chemotaxis is not a direct simple phenomenon, due to simple forces of attraction and repulsion, is in itself of importance.

The foregoing study of laws of chemotaxis in Paramecium, while it is believed to contain some results that are new and significant, is not put forth as in any sense exhaustive. Certain laws came forth clearly, while certain lines of investigation proved comparatively unfruitful; these I have set forth for the positive value of the one and the negative value of the other. The work has been done chiefly on inorganic compounds; almost the whole field of organic compounds remains to be examined. Certain facts in the reactions toward inorganic substances remain at present isolated, not falling under any of the general laws above given; thus certain experiments seem to show that there is a strong repulsion exhibited toward iodine, bromine, and chlorine when uncombined, — though in the ionic condition these elements are purely attractive. The effect of metals other than the alkalies and alkaline earths, when uncombined with a strong acid, is of interest, and has not been determined. Finally, an examination of the specific physiological effects of chemical compounds on the different parts of the motor reaction, as well as on the substance and the general functions of the animals, would be of fundamental value. Such a study would not be directed purely upon one aspect, as upon repulsion and attraction, which are really the resultant of complex factors, but should have in view any change produced by the substances studied

SUMMARY AND CONCLUSIONS.

In the introduction to the first of the Studies of which this is the fourth, the program for work was given as the investigation of the life activities of a single unicellular organism as completely as possible, in the hope that when such a study should have been carried to some degree of completeness, it should furnish a key to the understanding of the activities of other organisms, and should throw light on the essential nature of chemotaxis and similar phenomena. The investigation of the activities of Paramecium thus begun is far from being complete, yet I believe it may be said that the results thus far gained have already justified the plan of work. The mechanism of attractions and repulsions has been made out, and the third paper of the series shows that this furnishes at once a key to the understanding of

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the activities of certain other infusoria (Spirostomum and Stentor). Studies now in progress show that it throws equal light upon the activities of other, more distantly related, organisms. All the Studies thus far published have dealt more or less with chemotaxis, and in concluding the present paper upon that subject, it will be well to summarize in the form of brief statements the important conclusions in regard to the phenomena which have gone under this name, drawing upon the previous Studies as well as the present one for this purpose and emphasizing some important points which have not been emphasized elsewhere.

1. The motor reaction by means of which chemotaxis is brought about is identical with that produced by mechanical shock or any other stimulus, so that chemotaxis is not an activity differing *in kind* from others. This motor reaction is as follows: the animal swims backward, turns toward one side which is structurally defined (the aboral in Paramecium), then swims forward.

2. This motor reaction is the same, whatever the position of the chemical or other stimulus, or the part of the body of the animal that it acts upon, so that the same chemical which when acting on the anterior end of the animal causes it to move away, causes it when acting upon the posterior end to approach. The direction of motion has no relation to the localization of the stimulus. The usual relation of the direction of motion to the position of the stimulus depends upon the fact that owing to the forward motion, stimulation almost universally occurs at the anterior end.

3. Negative chemotaxis or repulsion toward any substance is due merely to the fact that this substance sets in operation the one motor reaction above described. The Paramecium then does not come to rest until the random movements thus caused have carried it by chance, as they must in time, into a region containing none of the substance which causes the motor reaction.

4. Different chemicals have different specific effects upon the various component parts of the above reaction; one part may be intensified or weakened, while other parts are modified in some different way. The motion to or from the chemical is the resultant of these specific effects taken together. The direction of motion, known as positive or negative chemotaxis, is therefore a complex phenomenon, resulting indirectly from various factors.

5. Paramecia are nearest the quiet condition when in a weakly acid solution. Paramecia at rest under normal conditions are in such

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a weakly acid solution, due to the carbon dioxide in the water, produced by themselves.

6. When Paramecia in such a weakly acid solution come in contact with a solution containing no acid, the motor reaction (with its reversal of cilia) is produced, so that the Paramecia do not pass out of the acid solution.

7. On passing from a neutral solution to a weakly acid solution no reaction is caused, but if now the Paramecium attempts to pass back again out of the acid solution, the reaction is produced (as in 6), so that the animal remains in the acid solution. The acid thus changes the physiological condition of the Paramecium, so that it now reacts to the neutral fluid.

8. The anions or acid ions present in salts tend to throw the Paramecia into the same physiological condition as the pure acid, so that the Paramecia afterward react when they come in contact with a fluid not containing these ions.

9. It results from 5, 6, 7, and 8 that Paramecia collect in solutions having a weakly acid reaction, or in solutions of salts in which the anion is especially active. Thus the so-called positive chemotaxis of Paramecium is brought about. The positive chemotaxis toward certain nutritive fluids, such as meat extract, is due to the acid reaction of the latter, not to its nutritive value.

10. Alkaline solutions and solutions of salts containing the kations of the alkali metals or of the earth alkali metals produce in a marked degree the motor reactions of Paramecium. As a result the Paramecia do not come to rest until their random movements have brought them into a solution which does not contain a large proportion of these ions. Thus the apparently negative chemotaxis of Paramecia toward these substances is brought about. In a series of salts of any one of the halogens with the different alkali metals, the power of producing the motor reaction (and hence the repellent power) decreases as the atomic weight increases, *except* that potassium salts are throughout more repellent than the lighter sodium salts.

11. In solutions of salts containing the kation of a strong alkali metal, as well as a powerful anion, as of fluorine or chlorine, both of the ions may act on the Paramecia, — the former causing the motor reaction when the Paramecium attempts to enter the solution, the latter when it attempts to leave it. In this case the Paramecia gather in a ring in the outer margin of a drop of the solution and are apparently both repelled and attracted by it.

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12. The chief factor causing the motor reaction which results in negative chemotaxis is not the injuriousness of the substance, but is of a chemical nature. Two substances of equally injurious properties have by no means equal repellent powers, but those which are alkaline or contain kations of the alkali or earth alkali elements are much more repellent than most others, without regard to injurious effects.

13. Nevertheless, any substance which directly and severely injures the Paramecia does thereby cause a motor reaction. But this reaction is not produced till the injury has occurred and it is too late to save the Paramecia. They therefore swarm into chemical substances of certain sorts by which they are immediately killed.

14. It is therefore possible to distinguish (1) reactions due purely to the chemical nature of the substance, the reacting Paramecia being entirely unharmed, and (2) reactions due purely to the injury produced by the substance, without regard to its chemical nature. Reactions of the latter class are not sufficiently precise to be protective.

The investigation of which the results are presented in the foregoing paper was pursued in the Laboratory of the United States Fish Commission for the Biological Survey of the Great Lakes, at Put-in-Bay, Ohio, in the summer of 1898, and continued at Dartmouth College during the following autumn. I desire to express my sincere thanks to the officials of the U. S. Fish Commission and to Professor J. E. Reighard, Director of the Survey, for courtesy and assistance in every way during the progress of the work.

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