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THE REACTION OF PROTOPLASM IN RELATION TO CHEMIOTAXIS.*

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THE present paper deals with the influence exerted upon cell protoplasm by acids and alkalies acting under conditions which imitate or exaggerate those under which the phenomena of chemiotaxis are produced. If any relationship exists between chemiotaxis, in respect of acids and alkalies, and the staining and chemical reaction of protoplasm, it appeared not improbable that it would be found on further investigation to be a determining factor in chemiotaxis, and to throw light upon the essential nature of this phenomenon. As the work progressed, it was found that it extended beyond the original scope of inquiry, but it has been thought better to publish an account of the investigation in its present form, apart from its later developments, which are not yet ended. Only such experiments as have been made in respect of paramoecium aurelia are here described, and only such changes produced by acids and alkalies as affect living protoplasm come within the range of investigation.

The term reaction in the strictly chemical sense refers to the presence of free acid or alkali, and indicates the relative preponderance of hydrogen or hydroxyl ions respectively in the fluid tested. In an acid it is to the concentration of the H^+ ions that the potentiality of the fluid for catalytic action is due, and similarly, an alkaline fluid is strong or weak according as the concentration of its OH^- ions is great or small. The chemical reaction of protoplasm was investigated by means of indicators, use being made of the results arrived at by Friedenthal.¹

In the second meaning in which the term reaction is employed we are dealing with a different phenomenon, namely, the action of so-called acid and basic dyes, a phenomenon dependent upon the side groups which the molecules of stainable tissue constituents possess. When the latter have salt-building side chains which react with dyes possessing an opposite acid or basic character, then the tissue constituents themselves are described as basic or acid. Reaction of protoplasm in this sense can be investigated only when such staining processes are employed as can be regarded as of the nature of a chemical reaction between two salts. Purely physical staining methods cannot be employed.²

THE DETERMINATION OF STAINING REACTION.

The basic (oxyphile) and acid (basophile) character of cell protoplasm was studied by means of methylene-blue-eosin,³ which affords a delicate test of the reaction of tissue constituents, and offers a much simpler means of differentiating between acid and basic characters than the successive application of methylene blue and eosin. Moreover, the use of methylene-blue eosin, consisting of a salt possessing specific characters and not being a mixture of two dyes, renders it certain that the resulting red and blue staining are brought about by a chemical combination of cell constituents with the acid and basic radicals of the dye, a purely physical explanation of the differentiation obtained being excluded.

* This work is here given in abstract. The full paper will be found in the *Zeitschrift für allgemeine Physiologie*, 1904, 4, 87-104.

† Methylene-blue-eosin is formed by the reaction of two molecules of methylene blue (tetramethyldiaminodiphenylamin)

with one molecule of eosin (potassium salt of tetrabromfluorescein)

The Action of Acids.

The action of acids upon the cell protoplasm of paramoecium differs in its effect in respect of the staining reaction according as the concentration employed is the minimal lethal concentration or is of considerably greater strength. If a paramoecium is placed in a solution of hydrochloric, nitric, sulphuric, tartaric, formic, lactic, citric, oxalic, carbonic, or boric acid, sufficiently dilute to kill in ten to thirty minutes, and is then washed in distilled water, it stains with methylene-blue-eosin like a normal paramoecium. There is no intensification of the blue staining, nor is any tendency exhibited by that portion of the cell which normally stains red to take on a purple tint, nor do any of the constituents of the cell which are achromophile become coloured. Thus dilute acids, the duration of whose action is limited to the time necessary to kill the paramoecia, do not alter the staining reaction. Repeated experiments have shown that no difference can be recognized between stained specimens of paramoecia, so killed, and paramoecia untreated with acids.

If, however, a stronger concentration of acid is employed for a somewhat longer period of time, then a different result is obtained. To produce this a 0.02N concentration of sulphuric, nitric, or hydrochloric acid must be used. A solution of a weaker acid of greater molecular concentration may also be employed, but it is not quite so effective. The protoplasm of paramoecia, so treated, when stained with methylene-blue-eosin, exhibits the change familiar to histologists in tissues whose staining characters have been "accentuated" by acids. The nucleus stains deeply blue, and the cell protoplasm takes on a diffuse blue or purple colour close to the periphery of the cell. In the cytoplasm a slight tendency to granularity is observable, but the oxyphile granules, normally present, are not recognizable. The cell pellicle, trichocysts and cell crystals, normally unstained, are unchanged in aspect. The cilia, which remain free from colour, are frequently unrecognizable; sometimes they are so changed as to be individually indistinguishable, but are fused together so as to form a broad layer round the periphery of the cell. The change induced by the prolonged action of acids, which persists after thorough washing in distilled water and in alcohol, consists therefore in an intensification of the normal combination with the blue radical of the dye, while the cell constituents which normally stain red become purple or bluish in colour, the basic radical of the dye tending to replace the acid radical.

The Action of Alkalies.

The influence of alkalies in respect of the staining reaction of the protoplasm of paramoecia differs according as the action is or is not limited to that affecting living protoplasm only. Thus if the hydrates of sodium, potassium, lithium, calcium, strontium, barium, or ammonium are employed in strength sufficient to kill paramoecia in ten to thirty minutes, and the organisms before disintegration takes place are rapidly washed in alcohol, and then stained with methylene-blue-eosin no definite change in staining reaction is observed. The behaviour of protoplasm in respect of alkalies of minimal lethal concentration is therefore similar to that of acids under the same conditions. No tendency to intensification of the redstained constituents of the cells is present, nor does basophile material show any diminution of staining capacity.

A marked alteration in staining reaction is produced by the prolonged action of a solution of sodium or potassium hydrate considerably above the minimal lethal concentration. If paramoecia are put for two hours into a 0.02N solution of alkali, to which 50 per cent. or more of alcohol has been added in order to prevent the occurrence of disintegration, and are then thoroughly washed in alcohol, the organisms, on staining with methylene-blue-eosin, exhibit a weakening of the combination of the cell constituents with the basic radical of the dye, while the combination with the acid radical is intensified. Thus the basophile material in the cell is but faintly tinted blue, the nucleus and basophile granules being indistinct, while the cell protoplasm takes on a diffuse red tint. The staining characters of the rest of the cell remain negative. In other words, an "accentuation" of acid staining occurs, forming the complement of that produced by the action of strong acids.

The Action of the Constant Current.

It is a point of considerable interest to ascertain how far the staining reaction of paramoecium, changed by the action of the constant current, is in harmony with the staining response of this organism when submitted to the action of acids and alkalies. Verworm⁴ attributed the galvanotaxis

of paramoecia to an attempt to escape from a stimulus applied to one end of the organism. Loeb and Budget¹ attempted to carry this explanation a step farther, putting forward the hypothesis that the constant current produces a development of acid and alkali at the pointed and swollen extremities of the organism respectively.

It was found when paramoecia were submitted to the action of the constant current until the cathodic end had become swollen and was commencing to disintegrate, while the anodic end had become pointed, that on subsequent treatment with methylene-blue-eosin, the staining reaction of the organism was only affected quantitatively in so far as it was weakened or strengthened by the swelling and condensation of the cell protoplasm, but that no qualitative change occurred. At the pointed end the oxyphile granules were numerous and the cytoplasm appeared deeper in colour, while at the swollen extremity these granules were separated by fluid and the tint of the cytoplasm was correspondingly lighter. The staining of the basophile constituents of the cell protoplasm and nucleus was similarly unaltered. The constant current thus resembles that of acids and alkalies in minimal lethal concentration in the negative character of its influence upon the staining response of the protoplasm of paramoecia.

Before concluding this section the following commentary on the preceding observations may be offered.

In considering the relation of chemiotaxis, in respect of acids and alkalies, to the staining reaction of cell protoplasm, we are concerned only with such action as is exerted upon living protoplasm. When, however, acids and alkalies considerably above the minimal lethal concentration are employed, their action is no longer exerted solely upon living, but, on the contrary, is essentially exerted upon dead protoplasm, and this action has no direct bearing upon chemiotaxis. In this latter process, that is to say in the prolonged action of strong acids and alkalies upon paramoecia, producing an intensification of basic and acid staining respectively, a chemical combination of protoplasm with acids and alkalies occurs which influences the affinity for dye radicals of such of the constituents of the cell whose molecules bear appropriate haptophore side groups. When, on the other hand, acids and alkalies of minimal lethal concentration act upon living protoplasm, the condition of the haptophore groups remains unaltered. In this case, either no combination of protoplasm with acid and alkali occurs, or, if such takes place, it must be of a different character to that exerted by strong acid and alkali upon dead protoplasm.

If instead of applying staining methods to dead protoplasm, living protoplasm is stained, the results obtained are similar to the above described. Thus, when paramoecia are put into a dilute solution of methylene-blue-eosin, intra-vitam staining with the basic radical of the dye takes place. If previous to staining the organisms are placed for some hours in dilute acid or alkali insufficient to cause death, it is then found that the surviving organisms stain in the same way as paramoecia untreated with acid or alkali.

THE CHEMICAL REACTION OF PARAMOECIUM.

As already stated, the chemical reaction of paramoecium was studied with the aid of indicators, these, from their ease and rapidity of application, possessing advantages of considerable moment in dealing with material so liable to change as is living protoplasm. The delicacy with which observations can be made by means of indicators is, however, much less in the case of cell protoplasm than in colourless fluids. By actual trial it was found that only a small thickness of cell protoplasm (obtained by centrifugalizing paramoecia) could, owing to the opacity of the protoplasm, be studied in respect of colour. Further, by using acid and alkaline fluids, coloured by the indicators employed, and observed in the same thickness as the protoplasm under consideration, it was found that the limit of colour reaction which was recognizable with certainty when phenolphthalein and methyl-orange were used, was represented by a concentration of not less than 1×10^{-3} H⁺ or OH⁻ gramme-ion per litre. In addition to the above mentioned indicators, congo-red, lacmoid, litmus, sodium sulphalizarinate, neutral red, rosolic acid and B-nitrophenol were employed, but the first two were found to be the most useful. Too much indicator must not be used, since there is then some risk of the tint changing as the result of mass action.⁶

When paramoecia are put into a 0.0001 N solution of methyl-orange for periods of time extending up to twenty-four hours, no red tint is observable in the organisms on

subsequent centrifugalization. In a solution of phenolphthalein of the same concentration paramoecia cannot be kept alive so long, owing to the presence of alcohol necessary to keep the indicator in solution, but, during the period preceding death, no red tint is developed. If the indicators are used in 0.001 N concentration, there is still no appearance of colour. From these experiments it follows that the reaction of the living protoplasm is less than that of a 0.001 N concentration of H⁺ or OH⁻ ions.

The Action of Acids.

If paramoecia are immersed in hydrochloric, nitric, or sulphuric acid of 0.001 N concentration, in which they will usually live for periods of five to thirty minutes, and before death occurs are rapidly removed by centrifugalization, washed in distilled water, and transferred to a solution of methyl-orange, no red tint is observable in the protoplasm, though the liquid in which the organisms are contained may, as the result of imperfect washing, assume a red colour. From this experiment it may be inferred that either acid is not absorbed by living protoplasm, or that if it is taken up it does not exist in the free state or in the form of a loose, reversible combination, but, on the contrary, forms a firm, irreversible combination. These observations can only be made with strong acids; with weak acids methyl-orange is not available.

The Action of Alkalies.

When similar experiments are made with the bases employed in the previous section, phenolphthalein being employed as indicator, the protoplasm of paramoecia fails to assume a pink tint, so that here again, as when acids are used, it is permissible within the limit of observation given by the indicator to infer that the alkali either enters into a firm combination with protoplasm or is catalytic in its action, but that no loose combination occurs.

The Action of the Constant Current.

A number of experiments were performed to investigate the action of the constant current upon the chemical reaction of paramoecia. The usual method employed consisted in passing the constant current through a solution of methyl-orange or phenolphthalein, and then observing the appearance of the paramoecia singly under the microscope or in bulk after centrifugalization. The experiments were varied by soaking dead paramoecia in methyl-orange or phenolphthalein, and then submitting them to the action of the current. In each case no evidence was obtainable of the presence of free or loosely-combined acid or alkali in the protoplasm of paramoecia either when examined in bulk, or when the pointed and swollen ends of the organism were observed under the microscope.

SUMMARY.

1. Exposure of paramoecia to acids or alkalies of concentration sufficient to kill in twenty to thirty minutes does not affect the staining reaction of the organisms.

2. Prolonged exposure of paramoecia to acids or alkalies of higher concentration causes the well-known appearance of "accentuation" of basic and acid staining respectively, but this is a change affecting the protoplasm after death, and therefore of a different order from that occurring in relation to chemiotactic phenomena.

3. Paramoecia killed by the constant current resemble paramoecia killed by acids and alkalies of minimum lethal concentration in respect of the absence of any alteration of staining reaction.

4. The chemical reaction of the protoplasm of paramoecia cannot be determined by means of indicators in respect of a concentration of H⁺ and OH⁻ ions, which is less than 0.001 N. Within this limit the protoplasm, living or dead, gives no evidence of acid or alkaline reaction.

5. The protoplasm of paramoecia killed by mineral acids or by alkalies of low concentration gives no evidence of acid or alkaline reaction with methyl-orange or phenolphthalein.

6. Paramoecia killed by the constant current do not exhibit any acid or alkaline reaction.

In conclusion, I gratefully express my indebtedness to Professor Max Verworn, in whose laboratory this work was carried out, for his kind assistance and advice throughout the course of the investigation.

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