SCIENTIFIC GRANTS COMMITTEE

OF THE

British Medical Association.

REPORT XCI.

THE ACTION OF ACIDS AND ALKALIES UPON
LIVING PROTOPLASM.

By J. O. WAKELIN BARRATT, M.D., B.Sc.LOND.,

British Medical Association Research Student.

[From the Physiological Institute, University of Göttingen.]

In a previous communication¹ it was shown that acids and alkalies in a concentration insufficient to cause death do not produce any recognizable alteration in the staining reaction of the protoplasm of paramoecia, and that in order to change the staining reaction of these infusoria, it is necessary to act upon them after death with relatively concentrated acids and alkalies. In the present research, the nature of the action of acids and alkalies upon the living protoplasm of paramoecia has been further studied, and in particular the problem has been investigated whether these substances are used up, as in chemical reaction, or merely act catalytically, for example, like hydrochloric acid, when employed for inverting cane sugar.

At the outset of this inquiry it was necessary to ascertain the lethal concentration² of the acids and alkalies employed (Table I).³ On comparing their action it is seen that some of the weaker acids (formic, lactic, oxalic) are more poisonous than the strong mineral acids, hydrochloric, nitric, and sulphuric acids; while, on the other hand, the extremely weak acids (carbonic, carbolic, boric, and hydrocyanic acids) are considerably less poisonous to the protoplasm of paramoecium. Similarly the strong alkalies (potassium and sodium hydrate) are less poisonous than the weak base ammonium hydrate, and considerably more poisonous than the extremely weak base anilin. On an examination of the ionic concentration which reveals still greater divergencies between different members of the series,⁴ it becomes at once apparent that the action of acids and alkalies is not a simple

TABLE I.—The Lethal Concentration of Acids and Bases.

	Concentration Employed.	Paramoecia Killed at end of
Hydrochloric acid ...	0.0002 <i>n</i>	7 minutes.
Nitric acid ...	0.0002 <i>n</i>	15 "
Sulphuric acid...	0.0002 <i>n</i>	17 "
Formic acid ...	0.0001 <i>n</i>	15 "
Lactic acid ...	0.0001 <i>n</i>	22 "
Oxalic acid ...	0.0001 <i>n</i>	7 "
Tartaric acid ...	0.0001 <i>n</i>	12 "
Phosphoric acid ...	0.0001 <i>n</i>	15 "
Citric acid ...	0.0001 <i>n</i>	13 "
Acetic acid ...	0.0002 <i>n</i>	30 "
Carbonic acid ...	0.0140 <i>n</i>	23 "
Carbolic acid ...	0.0100 <i>n</i>	23 "
Boric acid ...	0.4500 <i>n</i>	5 "
Hydrocyanic acid ...	0.3000 <i>n</i>	12 "
Potassium hydrate ...	0.0030 <i>n</i>	14 "
Sodium hydrate ...	0.0030 <i>n</i>	15 "
Lithium hydrate ...	0.0020 <i>n</i>	10 "
Calcium hydrate ...	0.0020 <i>n</i>	10 "
Strontium hydrate ...	0.0010 <i>n</i>	23 "
Barium hydrate ...	0.0010 <i>n</i>	30 "
Ammonium hydrate...	0.0010 <i>n</i>	2 "
Anilin ...	0.0400 <i>n</i>	15 "

¹ The Reaction of Protoplasm in Relation to Chemiotaxis. BRITISH MEDICAL JOURNAL, 1904, 1, p. 1413.

² Proc. Roy. Soc. Lond., 1904, LXX, p. 100.

³ The concentration in this table is expressed in gram equivalents per litre, except for carbonic and boric acids, whose concentration represents gram molecules per litre.

⁴ Fuller details will be found in Die Wirkung von Säuren und Basen auf lebende Paramoecien, Zeit. f. Allg. Physiol., 1904, Bd. 4, S. 438.

catalytic action, dependent upon ionic concentration, and it is therefore to be anticipated that a chemical reaction takes place, in the course of which acid and alkali are used up.

In order to determine this point, paramoecia, after thorough washing in distilled water, were placed in a dilute acid or alkaline solution (in which most of the infusoria remained alive at the conclusion of the experiment), and the conductivity of the solution, as indicated by the deflection of a sensitive galvanometer when a fixed potential difference was established between two platinized platinum poles immersed in the liquid, was from time to time noted. It was found that a fall of conductivity occurred after the addition of the paramoecia, while when a control experiment was performed without any paramoecia, no fall or only a relatively slighter fall of conductivity took place. It follows therefore that when acids or alkalies act upon the living protoplasm of paramoecia they are used up in the process. On the assumption that the fall of conductivity is entirely dependent on the amount of acid or alkali disappearing from solution, the quantities of these substances entering into reaction with living protoplasm⁵ are calculated in the third column of Table II in terms of the weight of the protoplasm employed, the latter being ascertained in each experiment by counts of

TABLE II.—The Disappearance of Acid and Alkali from Solution, Brought About by the Agency of Living Paramoecia.

	Concentration Employed.	Equivalent Absorption of HCl or KOH, expressed in Percentage Terms of the Weight of Protoplasm Employed for Experiment.
Hydrochloric acid ...	0.00015 <i>n</i>	0.012 per cent.
Nitric acid ...	0.00030 <i>n</i>	0.0.6 "
Sulphuric acid...	0.00015 <i>n</i>	0.023 "
Formic acid ...	0.0001 <i>n</i>	0.022 "
Lactic acid ...	0.00010 <i>n</i>	0.042 "
Oxalic acid ...	0.00010 <i>n</i>	0.039 "
Tartaric acid ...	0.00010 <i>n</i>	0.002 "
Phosphoric acid ...	0.00010 <i>n</i>	0.013 "
Citric acid ...	0.00010 <i>n</i>	0.020 "
Acetic acid ...	0.00010 <i>n</i>	0.007 "
Potassium hydrate ...	0.00100 <i>n</i>	0.020 "
Sodium hydrate ...	0.00100 <i>n</i>	0.100 "
Lithium hydrate ...	0.00100 <i>n</i>	0.320 "
Calcium hydrate ...	0.00100 <i>n</i>	0.560 "
Strontium hydrate ...	0.00100 <i>n</i>	0.440 "
Barium hydrate ...	0.00100 <i>n</i>	0.320 "

the paramoecia employed, aided by haemocrit measurements. The numbers thus obtained, which represent the amounts of acid and alkali reacting in the individual experiments performed, and not the maximum amounts which can react upon living protoplasm without causing death during the period of experiment, are probably in reality smaller than those actually obtaining, since, as will presently be shown, there is reason to believe that the protoplasm, when injured by acids or alkalies, gives up salts to the surrounding fluid and thus adds to its conductivity.

This source of inaccuracy was avoided by making a further series of experiments, in which the above observations were repeated with the aid of a palladium-hydrogen concentration element, in one limb of which a simple dilute acid or alkaline solution was employed; while in the other limb, to a dilute acid or alkaline solution of the same concentration, a definite weight of paramoecia, previously carefully washed in distilled water, was added. The difference of concentration at the conclusion of the experiment, when the paramoecia were still for the most part living, was calculated, for hydrochloric acid, from the formula:

$$E = 0.096 \log \frac{a}{b},$$

which also serves, with a change in the value of the constant,

⁵ In order to permit of comparison of the molecular amounts of acid and alkali entering into combination with protoplasm, the former are calculated in terms of the equivalent amounts of hydrochloric acid and the latter in terms of the equivalent amounts of potassium hydrate. In this way differences depending on the considerable variation of molecular weight of different members of the two series disappears. The duration of the period of experiment, it may be observed, varied from ten to twenty minutes, and the number of paramoecia still living at the close of the experiment from 85 to 97 per cent.

for the calculation when sulphuric acid, or potassium or sodium hydrate are employed. In this formula E represents the potential difference of the element, which is measured by Poggendorff's compensation method; a is the original concentration of the acid or alkaline solution employed; b the concentration at the close of the experiment of the solution in which the (still living) paramoecia are contained. Without entering into further details of the method employed and the many precautions necessary to ensure accurate observations, which are given at length elsewhere,⁶ some of the results obtained may be here considered. These, which are given in Table III,

TABLE III.—Amount of Acid and Alkali Disappearing from Solution in Per Cent. of the Weight of the Paramoecia Employed.

	Concentration Employed.	Amount of Acid or Alkali Withdrawn by 100 Parts of Protoplasm.
Hydrochloric acid ...	0.00067n	0.30 HCl
Sulphuric acid..."	0.00067n	0.13 H ₂ SO ₄ [= 0.16 HCl]
	0.00067n	0.24 " [= 0.18 "]
Potassium hydrate ...	0.000330n	1.95 KOH
	0.000330n	0.74
Sodium hydrate ...	0.000670n	1.24 NaOH [= 1.74 KOH]
" "	0.000570n	1.52 " [= 2.11 "]

confirm the results obtained by the former method, and show, further, that living protoplasm may react with still larger amounts of acid and alkali than are indicated in Table II, reaching in fact during the period of experiment, which varied from eight to forty minutes, as much as 0.3 per cent. in the case of hydrochloric acid and 2 per cent. in the case of potassium hydrate.⁷ With palladium-hydrogen electrodes, however, only the H⁺ ions of the acids and the OH⁻ ions of the alkalis are shown to be used up, and no information is afforded of the part played by the remaining ions. Only in the case of hydrochloric acid has this point been investigated, and condition of the solution employed in respect of the Cl⁻ ions pre-studied. To this end a series of observations were made with calomel electrodes, the arrangement adopted being :

and the potential difference measured being the sum of $E_1 - E_2 + E_3$ obtaining at the three surfaces of contact. From the observed potential difference, the initial concentration of the hydrochloric acid employed being known, the alteration of the Cl⁻ ionic concentration brought about by paramoecia can be calculated from a formula similar to the one given above. In this way the unexpected result was obtained that when paramoecia are added to a dilute hydrochloric acid solution, not only do Cl⁻ ions not disappear, but that an actual increase of these ions takes place. The cause of this increase was found on further investigation to be due to the discharge of chlorides by the protoplasm of paramoecia, when injured by the acid, into the surrounding fluid.

Summary.

By the above-described methods of investigation it is shown (1) that the action of acids and alkalis upon living paramoecia is not a catalytic action, but that (2) a chemical reaction takes place in which H⁺ and OH⁻ ions disappear. Further, it has been shown (3) that when dilute hydrochloric acid acts upon living paramoecia an increase of the Cl⁻ ions takes place.

In conclusion, I must express my indebtedness to Professor Verwor, in whose laboratory this work was carried out, and also to Professor Nernst, who kindly permitted me to make the physical measurements in the Physico-Chemical Institute in Göttingen, and to Professor Cohn, at whose suggestion palladium-hydrogen electrodes were employed.

⁶ Die Addition von Säuren und Alkalien durch lebendes Protoplasma, *Zeit. f. Allg. Physiol.*, 1905. Bd. 5, 8 10.

⁷ It is interesting to compare the amount of acid and alkali reacting with living protoplasm with that taken up by proteids. Cohnheim (*Zeit. f. Biologie*, Bd. 33, 1895, s. 439) found that albumose, protalbumose, deuteralbumose, and antipeptone combined respectively with 4.15 per cent., 4.32 per cent., 5.48 per cent., and 15.87 per cent. of hydrochloric acid; and Bugarsky and Liebermann (*Arch. f. d. ges. Physiol.*, Bd. 77, 1895, s. 51) observed that albumin and albumose combined with 2 per cent. and 4 per cent. respectively of their weight of sodium hydrate.

REPORT XCII.

ON THE ORIGIN AND LIFE-HISTORY OF THE INTERSTITIAL CELLS OF THE OVARY IN THE RABBIT.

By JANET E. LANE-CLAYPON,

British Medical Association Research Scholar.

[From the Physiological Laboratory, University College.]

THE majority of investigators agree in considering the germinal epithelium to be derived by a process of differentiation from the peritoneum. There is, however, considerable difference of opinion as to the means whereby the cells of this epithelium come to form part of the ovary, and as to the subsequent fate of the cells.

Pfäfer⁹ considered that the germinal cells grew down into the underlying mesoblast in the form of tubules (egg tubules), the cells thus invaginated giving rise by division to ova, and also probably to follicle cells, some of these latter being possibly derived directly from the germinal epithelium.

Waldeyer¹¹ thought the embedding of the cells was accomplished by the upgrowth of processes from the underlying mesoblast, cutting off groups of cells, which ultimately gave rise to ova (primordial ova) and follicle cells. De Foulis⁸ believed both upgrowths of mesoblast and downgrowth of cells to take part in the embedding of the cells of the germinal epithelium; he differs from the above-named and most observers in deriving the follicle cells from the mesoblast, a view only held by two other observers (Schrön¹⁰ and Wendeler¹³). Balfour⁴ describes the formation of "egg-clusters" by the processes of upgrowth of mesoblast and downgrowth of cells, the cells of the egg-clusters forming either (1) ova by a process of differentiation, and (2) follicle cells, there being a number of cells resembling follicle cells whose destiny he could not trace; he also describes the formation of protoplasmic masses by the aggregation of several primordial ova, of which he thinks one might survive at the expense of the rest. Bühler⁶ has described the streaming inwards from the periphery of germinal cells, which arrange themselves around the primordial ova, forming the follicular epithelium.

V. Winiwarter¹² has described the processes of nuclear transformation connected with ovogenesis. There are three types of cells present in the fetal ovary before birth—protobroque cells of varieties a and b , and deutobroque cells. The protobroque a cells are the original germinal cells; these divide, giving rise to more cells of the same class and also to cells protobroque b ; these in turn divide, giving rise to more cells of the class b , and also to deutobroque cells. These last are the large clear cells seen in the fetal ovary in considerable numbers.

Immediately after birth nuclear changes begin to take place in some of these cells, the ultimate result being a primordial ovum. The changes start in the cells most centrally situated, and pass gradually towards the periphery, the early stages being still seen in this part at the tenth day after birth.

The nuclear changes leading to ovogenesis he classifies into five stages :

1. The leptotenic stage : The chromatin is present in the form of fine filaments spread out over the nuclear area. This stage is very fugitive.
2. The synaptonic : Here the filaments are becoming gradually massed together until they are finally all collected, the chromatin showing as a solid mass lying at one side of the nuclear area.
3. The pachytenic : The chromatin becomes again spread out over the nuclear area, but this time in thick strands, which subsequently show a tendency to a double appearance, thus giving rise to the
4. Diplotenic stage : This is followed by the
5. Final or dielyate condition of the primordial ovum, where the chromatin is arranged in a more or less reticular form, and there are one or two lumps of chromatin as well.

The question of the formation of "ova" in later life is not one upon which the literature throws much light—it consists chiefly of isolated observations of the formation of follicles in ovaries of cats, rabbits, and women, either in the rutting season and at the menstrual periods, or shortly after parturition.

Amann¹ recently published an interesting case of a woman of 63 upon whom he performed double ovariectomy, the ovaries being in a condition of incipient cystic adenoma. He found all stages of ova and follicle formation, all derived by in-growth of the germinal epithelium. The interest of the