A COMPARISON BETWEEN THE ACTION OF CARBONIC ACID AND OTHER ACIDS UPON THE LIVING CELL

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With 2 Text-figures

The physiological action of CO_2 on the living cell is of great scientific interest and has been the subject of numerous researches. On one hand, this acid is elabotared by the cell in the course of the metabolic process, on the other, it forms the basis of the CO_2-bicarbonate buffer system, which is a most important one among the buffer systems of plant and animal cells, and in the media in which they live. Carbonic acid possesses besides a number of characters quite different from those of other acids. NIKITINSKY [1928 (35)] has shown, in his preliminary investigations on some aquatic animals, that if HCl and CO_2 are applied in an equal concentration (in percents), the toxicity of the latter is considerably greater than that of the former. It is also known that CO_2 inhibits the development of bacteria [SIERAKOWSKI and ZAJDEL, 1924 (48)], and the segmentation of the egg cell of certain marine organisms [CLOWES a. SMITH, 1923 (11); SMITH and CLOWES, 1924 (51)], but this phenomenon cannot be attributed solely to acidification, i.e. the effect of an acidification by a mineral acid, with the same pH, is considerably less. The specific action of CO_2 on the cardiac muscles of the turtle, as compared to the action of mineral acids, has been described by SMITH [1926 (50)]. The author sees the cause of the specific nature of the action in the greater permeability of the living cells as regards carbonic acid (this will be discussed further on). It has also been observed that carbonic acid stops the movement of the branchial cilia in *Mytilus edulis* [HAYWOOD, 1925 (22)], the movement of the leucocytes [PEARSE, 1925 (39)], and the contractions of the rabbits intestine [FRASER, 1925 (15)], pH being here much less acid than with mineral acid. A movement cut short by the action of CO_2 can be completely restored, if the subject is transferred into a medium without CO_2, whereas, after remaining in a mineral acid, it cannot be revived [FRASER (15)]. The reappearance of a movement interrupted by CO_2 has also been observed by LOPRIORE [1895 (32)] and others, in the protoplasm of the anthers of *Tradescantia virginica*. Likewise, in small doses, CO_2 can accelerate the movement of *Paramaecium*, whilst a mineral acid, with the same pH, does not do so [CHASE a. GLASER, 1930 (10)]. Frequently, this acid can cause an alteration in the phototaxis sign of aquatic organisms [LOEB, 1906 (30), BRUCHATOWA, 1928 (6)], whereas this has not been observed for other acids [BRUCHATOWA (6)]. Many phenomena have been reported demonstrating the specific influence of CO_2 the state of living protoplasm. JACOBS [1922 (27)] has shown by numerous examples that CO_2 influences the viscosity of protoplasm, by first diminishing and then increasing it, this phenomenon being reversible. Nothing of the kind was to be seen with the mineral acid (HCl). In this way, JACOBS interprets the interruption of the vacuolar pulsations of infusoria, as due to carbonic acid. A dissolution of the plasmalemma in an amoeba, has been observed after the introduction of bubbles of CO_2 [REZNIKOV and CHAMBERS, 1927 (43)], see also the dissolution of the *pellicula* of *infusoria* in the papers of JACOBS [1912 (23)], GALAGIEW and MALM [1931 (16)], and NIKITINSKY and MUDREZOWA-

Wyss [1930 (36)], also a swelling of the cells in *infusoria* [Jacobs (23), (27)], in the hyphae of fungi and in the pollinic tubes of Gymnosperms [Lopriore (32), Becker, 1933 (2)]. Probably all these phenomena are closely connected, partly with the acidifying action of CO_2 on the proteins of the living protoplasm, and partly with their reversible dissociation described by Adolf and Pauli [1924 (1)], which, according to the latter authors, cannot be ascribed exclusively to acidification. A great many investigators attribute the specific action of carbonic acid to its peculiar faculty of easily penetrating into the cell. This peculiarity of CO_2, demonstrated in the work of Smith cited above (50), has also been pointed out by Krogh [1919 (29)], who discovered that this gas penetrates through the membrane of the tissues 30 times more quickly than O_2. Jacobs [1920 (24, 25) and 1922 (26)], has shown with the corolla of flowers containing a natural indicator, anthocyan, with the eggs of the starfish stained with neutralred, and with the artificial cell prepared from frog skin, that the penetration into the cell of undissociated H_2CO_3, is much greater than that of other substances, especially of those strongly ionized. In this manner, CO_2 can acidify the cell even if it passes into it from a neutral or alkaline solution. Jacobs explains in the same way the sour taste of the alkaline bicarbonate solutions. Also, investigations were carried out by Chambers, which showed that the difference in permeability of the starfish egg to bicarbonate ions and carbonic acid is the function of only the surface layer of the protoplasm, and not of its inner layer, nor of the cell membrane, for, with a micro-injection of an alkaline solution of CO_2 as used by Jacobs, an alkalinisation was obtained, instead of the acidifying which occurred when the cell was placed in the solution [Chambers, 1922 (7)]. Indications of the greater ease of penetration of CO_2, as compared to mineral acids, are also to be found in the researches of Chase and Glaser (10), who studied *Paramaecium*, in those of Smith [1923 (52)] on the cells of the corolla of *Ipomoea*, and those of Wehrli Hegner and Wyss [1933 (57)], who studied the penetration of different acids through the living tissues of the blood vessels of the frog. Osterhout and Dorcas [1925 (37)], and later Jacques and Osterhout [1930 (28)], have shown in detail in *Valonia* that CO_2 penetrates into the cell chiefly as an undissociated acid (pH of the medium being about 5·0), and not as ions of bicarbonate (pH being about 7·0). According to Smith and Clowes (48), not only does the undissociated acid penetrate without any difficulty into the cell, but also the anhydride CO_2, as the latter especially can very easily be dissolved in the lipoids, whilst H_2CO_3, owing to its structure, ought to penetrate less vigorously into the cell. Many investigators relate the facility of penetration of CO_2 to its feeble dissociation. It has been indicated, as a general rule, that feebly dissociated acids and bases penetrate into the cell much more rapidly than strong acids, bases and their ions [see Harvey, 1911 (20), 1915 (21), Crozier, 1916 (13, 14), Malm, 1930 (33), Collander, Turpeinen and Fabricius, 1931 (12)]. There are indications also, however, of a considerable penetration of the latter [Loeb and Gilman, 1924 (31), Brooks, 1923 (4, 5)]. Lastly, Gompel [1925 (18)] does not relate the rapidity of penetration of the acids to either their dissociation or their solubility in lipoids etc. How can one explain this specific action of CO_2? Can the action of CO_2 on the cell be attributed solely to its property, as shown in the experiments of Jacobs and Chambers (24, 25, 26 and 7), of easily penetrating into the cell of there by acidifying[1] the cell juice and the protoplasmic inclusions[2], as is elaborated in the recent paper of Spek and Chambers [1933 (54)], which continues the researches begun earlier by Chambers and his co-workers [(18, 41, 44)]? Chase and Glaser explain thus the phenomena it produces (10). It is scarcely possible to explain thus all the different

[1] Another explanation of the tint of the indicator in the protoplasmic granular inclusions under the action of CO_2, which Spek (54) gives to this phenomenon.

[2] CO_2 and other acids are unable to acidify the hyaloplasm itself within physiological limits [see Chambers, 1928 (8), Reznikoff and Pollack (44), Pollack (41)].

kinds of phenomena in the living cell although there reactions certainly play an important part in the life processes of the cell. The observations of ADOLF and PAULI (1) favour the supposition that CO_2 enters into a mobile, reversible combination with the proteins, which cannot be due only to acidification. Also in the paper by SPEK and CHAMBERS (54), the peculiar behaviour of CO_2, in inducing a reversible phenomenon resembling the coagulation brought about in the protoplasm if an injection of other acids and salts with multivalent cations is made into the cell; the latter coagulation, however, is not reversible, as with CO_2 [see also the reversible alteration in the viscosity of protoplasm, by the action of CO_2, JACOBS (27)]. Further than this, a great many authors have pointed out the narcotic action of carbonic acid [see JACOBS (23), NIKITINSKY and MUDREZOWA-WYSS (36), GALAGIEW and MALM (16), LOPRIORE (32), FRASER (15), WINTERSTEIN, 1919 (58) and others], in which it resembles protoxide of nitrogen, ether and chloroform: but this effect can scarcely be ascribed to its acidifying action. If carbonic acid owed its specific action only to its faculty of easily penetrating into the cell, whilst within the cell its H-ion alone was active, we would be entitled to suppose that any other acid, which can penetrate as easily into the cell, as for instance valerianic acid, possesses the same property. Besides this, in an intracellular injection, the effect of any kind of acid ought to be the same as that of CO_2; however we do not see this, if we compare the experiments made with microinjections of HCl [REZNIKOFF and POLLACK (44)] and CO_2 [see REZNIKOFF and CHAMBERS, 1927 (43)], (where the CO_2 causes a specific dissolution of the plasmalemma, which does not take place with the HCl), nor do we find evidence of any such action in the researches of SPEK and CHAMBERS (54). If the properties of carbonic acid depend not only on the H-ion, but also on the anion, or rather the molecule of CO_2, entering into a reversible reaction with the proteins of the cell, as this does not occur with the anions and molecules of other acids, evidently, the phenomena thus produced will in many points differ sharply from the action due to those other acids which can easily penetrate into the cell.

In beginning my researches I was seeking for an answer to the following questions: 1) does the toxicity of CO_2 and of mineral acids really, and always, depend on the difference in the rapidity of penetration into the cell? 2) Is the toxicity of CO_2 due to the H-ion, and hence is the same pH necessary in order to obtain an equally effective action of CO_2, and of other acids? 3) Do those acids which penetrate easily into the cell have at the same time a narcotic action like CO_2, or is this a specific property of the latter? In order to answer these questions the objects for the experiments had to be so chosen, as to fulfil two principal requirements: 1) They must possess some indicator of the toxic or narcotic action of carbonic acid, such as for instance the cessation of movement of the entire cell, or of its protoplasm, 2) an indicator showing the pH in the cell, either by a natural coloration or else by the introduction of an artificial dye. Among the numerous objects tried for this purpose the most convenient appeared to be the cells of the hairs on the anther filaments of *Tradescantia virginica*, and *Paramaecium caudatum* stained by a solution of methylred.

I. EXPERIMENTS WITH TRADESCANTIA VIRGINICA

The object investigated, hairs from the anther filaments in *Tradescantia virginica*, contains a natural indicator-anthocyanin. This indicator possesses two colour transition points; from violet (the usual coloring of the flowers) into

dark blue pH 6·3, and from violet into red, pH being 4·3. The petals of *Tradescantia*, containing the same indicator, were boiled in buffer solutions of a determined pH, and in this manner a scale was prepared [see SMITH, 1933 (50)] with a transition at every 0·5 pH, as this indicator changes only within rather rough not strictly defined limits. With this indicator, according to the scale of the buffer solutions, a micro-scale was established. This scale was made with leaflets of the "Foliencolorimeter" of P. WULFF [1926 (59, 60)], stained by anthocyanin, concentrated by means of evaporation. The leaflets were placed in buffer solutions of different pH, where they changed colour. Then they were arranged into a scale, fixed on a round cover glass by means of a thin thread of canada balsam. This micro-scale was enclosed in canada balsam, because in it anthocyanin changes its colouring even more slowly than in the open air. Another cover glass was put over this and the whole was fastened by a metallic ring like the scale of the ocular-micrometer. Such a ring with a 1·9 mm. diameter, can be easily introduced into the ocular of any micro-scope [for a more detailed description of the micro-scale see BECKER, 1934 (3)]. In the conditions of my investigations this small contrivance has proved to be the most convenient one among all those used before for the determination of pH in the living cell. The R. I. M. method (Range Indicator Method), consisting of a comparison between the colour of the objects stained in different indicators [see SMALL (49), SCHMIDTMANN, 1924 (47), GICKLHORN and KELLER, 1926 (17), GUTSTEIN, 1932 (19), CHAMBERS and POLLACK, 1927 (9)] could not be applied here, as a number of indicators, owing to the difficulty of their penetration into the cell, were of no use, and only one which penetrated easily could be chosen (methyl red). The methods of PANTIN [1933 (38)] and REISS [1926 (42)], where a series of test tubes, containing a scale of buffer solutions, or a Bjerrums' wedge, are placed before the mirror of the microscope, and a coloured background is thus obtained, with which the investigated object is compared, and the method of ZACHAROWA [1925 (61)], where the scale is transferred to the eye piece by means of the mirror of the ABBÉ drawing apparatus, do not give any distinct colours, while with my micro-scale the colours are sufficiently clear for determination. Besides this the methods of PANTIN and REISS require the scale to be moved, which is very inconvenient for working, with a moving object (*Paramaecium*). In this respect, besides my micro-scale, the adaptation of VLÈS [1925 (56)] is excellent, but it is very complicated, and certainly cannot be made by hand, whilst the micro-scale with the leaflets of the "Foliencolorimeter" can be constructed by any one.

The investigation of the hair cells of *Tradescantia* was made, in the case of CO_2, in a drop of distilled water, by means of the Lopriore chamber (31), through which CO_2 was made to pass. With the other acids an object glass covered by a cover slip was used. Evaporation and alkalization in contact with the glass gave only a very slight error, which did not influence the experiment, as shown by control experiments, with a bath filled with diluted acid. In the case of volatile acids (CH_3COOH and valerianic acid), the researches were made, as in those with CO_2, with a Lopriore chamber (32). All the determinations of the pH in the solutions were made by means of the quinhydron

electrode of LAUTENSCHLÄGER's potentiometer. Three different series of experiments were carried out upon the hair cells of *Tradescantia*. The first series was made with somewhat strong solutions of acids (0·1—0·01 Mol., pH from 2·0—3·0), and saturated solutions of CO_2. This was required in order to ascertain whether the same degree of acidification of the cell sap, with each solution, is connected with an equal reaction; in these experiments, the cessation of the streaming movement of the protoplasm. The protoplasm itself allows the passage through it of the substances altering pH but does not itself show a change in pH before death ensues[1]. The pH of the cell juice was taken, when the movement of the protoplasm ceased entirely[2]. The mean pH was calculated, at which the movement of the protoplasm ceases in the investigated cells (see Table I).

Table I

Experiments on the relation between intracellular pH and total cessation of movement in the protoplasm. (Normal pH of the cell juice 4·3—4·9, mean 4·5)

	CO_2	Valerianic acid	CH_3COOH	Oxalic acid	Citric acid	H_3PO_4	HCl	H_2SO_4
Mean pH within the cell, when the movement of the protoplasm ceased .	4·5	4·22	4·05	3·84	3·64	3·59	3·90	3·80
pH of acid	3·8	2·79	2·98	2·67	2·05	2·00	2·25	2·10
Molarity of the acid . . .	0·03	0·1	0·1	0·01	0·1	0·01	0·01	0·01

As seen in table I, the movement of the protoplasm, when CO_2 was passed through, ceases when the colour of the indicator corresponds to the normal state of the hairs, as CO_2 is evidently unable to acidify the cell without at the same time inducing a noticeable change of pH (i.e. pH more acid than 4·3). As in the normal condition, the mean pH in CO_2 is equal to 4·5. Similar phenomena occur in valerianic acid, where the movement ceases before the cell has become sufficiently acidified, i.e. with a mean of 4·22 for pH. Analogous data have been obtained for acetic acid, however a somewhat stronger acidification is required in this case to arrest the movement (up to pH 4·05). The other acids, which are placed in the first group, i.e. HCl, H_2SO_4, H_3PO_4, citric and oxalic acids, when the protoplasmic movement is checked, induce a greater acidification, on the average between pH 3·6 and 3·9. From these results one can conclude that for a similar effect, namely cessation of the protoplasmic movement, it is not necessary that an equal quantity of H-ions should penetrate into the cell. On the contrary, to obtain the same effect by the action of CO_2 and by that of

[1] The fact that hyaloplasm does not acidify [indicated by CHAMBERS (8) and others in *Amoeba*], I succeeded in observing accidentally, on a hair cell, where the anthocyanin for some reason had partially penetrated into the protoplasm, and mixed with it. In the protoplasm the anthocyan was violet and inside the central vacuole it was violetish-red.

[2] The movement of the protoplasm does not cease at once. The streaming movement passes gradually into a quivering of the particles resembling the brownian movement, which by degrees becomes extinct.

the acids belonging to the second group (CO_2, CH_3COOH and valerianic acid) a much lower degree of acidification is required than with the acids of the first group. The processes within the cell, which eventually lead to cessation of movement, appear to the of the same type in each acid, but occur at different rates[1]. Thus, they all begin by a gradual diminution of the strands of protoplasm crossing the cell, till these disappear and a parietal layer is formed; the movement becomes slower and passes into the quivering "brownian" movement already mentioned. At the same time the principal masses of the protoplasm accumulate at both ends of the cell, in the shape of transparent masses, whilst the cell juice begins to change in colour. The nucleus, so far enveloped by the protoplasmic membrane, gets rid of it and becomes clearly visible. At a later stage, when the protoplasm ceases moving and begins to coagulate, little clots of chromatin become isolated in the nucleus and destruction of a caryorexis type sets in. Then the protoplasm gradually contracts into a lump, and the anthocyanin passing through it accumulates between the protoplasm and the cell wall, and frequently also in the form of a vesicle between the cell wall and the cuticle, causing the latter to swell, thus indicating its feeble permeability for the substances dissolved, in this case anthocyanin. (Such a separation of the cell walls has been frequently observed, especially with H_2SO_4, where, in consequence of the separation and of the rupture of the membrane, something resembling the caps of *Oedogonium* was formed.) On the whole it may be seen that the behaviour of *Tradescantia*, as regards permeability, is by no means the same with different acids. For instance CO_2, valerianic and acetic acids act very rapidly, penetrating at the same time into all the hair cells, whereas the acids of the first group penetrate much more slowly, the penetration in the hair proceeding upwards from the point of rupture. This fact is, indeed, further evidence of the considerable resistance of the cell membrane or the cuticle to penetration. However, it may be that here the penetration from the base upwards is also due to the injury sustained by the surface layer at the base of the hair, which has not had time to heal, when the hair is placed in the acid. Data on such an elective penetration of mineral acids through a rent of the tissue, are to be found in SMITH's work (43). The next series of experiments was made with solutions of acids, where pH was equal to the pH of distilled water, saturated with CO_2, i.e. 3·8. pH was determined electrometrically by the quinhydron method with the potentiometer of LAUTENSCHLÄGER. The duration of the experiments was as much as 3 hours. The results are as follows. The acids of the first group [H_2SO_4 (0·00022 Mol.), HCl (0·00025 Mol.), H_3PO_4 (0·00055 Mol.), oxalic acid (0·0002 Mol.) and citric acid (0·0004 Mol.)] pH being 3·8, did not check the movement of the protoplasm nor even hinder it, but rather they stimulated it, during the whole 3 hours that the experiment lasted. In H_2SO_4 only, towards the end of the experiment, in those cells where the outer layer of the membrane began to separate in the shape of small caps, the current became somewhat slower. The results with acetic, valerianic and carbonic acids are summarised in Table II.

[1] The only differences being that in CO_2 the period of the "brownian" movements is considerably shorter, and less noticeable than in other acids.

Table II

Comparative toxicity of CO_2, acetic and valerianic acid, pH of the medium being the same. (Numbers in percent of the total number of cells)

	Time of experiment before observation	Cessation of movement of the protoplasm in CO_2	"Brownian" movement	Normal streaming movement
CO_2 0·03 Mol. pH 3·8	25′	7	—	93
	50′	48	—	52
	40′ in CO_2 and 1 hour, after air replaces CO_2	—	—	100
	1 hour	37	—	63
	3 hours	100	—	—
	1 hour in CO_2 and 1 hour after air replaces CO_2	—	—	100
	3 hours in CO_2 and 1 hour after air replaces CO_2	40	1	58
Valerianic acid 0·01 Mol. pH 3·8	20′	50	10	40
	40′	50	15	35
	1 hour	52	14	34
	3 hours	73	27	—
Acetic acid 0·003 Mol. pH 3·8	20′	—	—	100
	40′	6	2	92
	1 hour	4	3	93
	3 hours	12	5	83

It is shown that the action of these three acids is much stronger than that of the acids of the first group. Acetic acid is the least active one among them; in it the movement ceases only in 12 % of the cells. Carbonic and valerianic acids are very much alike in the action they produce. It was not possible to record, the alteration of pH in the cell juice, in this experiment, as the acidity of the medium was not strong enough to change the colour of the anthocyanin. These experiments show that there is a distrinct difference between the acids of the first and of the second group, which approaches CO_2, but here, the difference of their penetrability through the cell membrane and the superficial layer of the protoplasm is not eliminated. Therefore on the grounds of this experiment no direct conclusions can be drawn, as to the difference in toxicity of these acids. Finally, a third series of experiments was undertaken with *Tradescantia* using carbonic, valerianic and acetic acids, in order to investigate their narcotic action upon the cell. The experiments were carried out in the following manner: the hairs were placed in a saturated solution of CO_2 (0·03 Mol. H_2CO_3)[1] or

[1] An error took place here in the calculation of molarity as it was made by taking H_2CO_3, whilst, according to TIEL and STROHECKER [1914 (51)], in a water solution carbonic acid will for the most part be in the form of CO_2, and the narcotic action belongs apparently to the anhydride itself. Thus the molarity of a CO_2 solution, calculating with a 2 % solution will be somewhat larger, though this does not influence our deductions.

in isomolar concentrations of valerianic and acetic acids (as it can be assumed that the toxic action belongs to the undissociated molecule and not to the H-ion), pH of the solutions being about the same. After a definite time (one hour), the number of cells in which movement of the protoplasm had ceased was counted, the solution of acid was replaced by water, and instead of CO_2 air was introduced through the chamber. An hour later, observations were again made. The results are given in Table III.

Table III

Investigation of the narcotic action of CO_2, CH_3COOH and valerianic acid on the hairs of Tradescantia. (Numbers expressed in percents for the total number of cells)

	Duration of experiment before observation	Cessation of movement of the protoplasm	Brownian movement of the protoplasm	Normal streaming movement of the protoplasm
CO_2 0·03 Mol. pH 3·8	1 hour	37	—	63
	1 hour in CO_2 and 1 hour after air replaces CO_2	—	—	100
Valerianic acid 0·03 Mol. pH 3·3	1 hour	52	33	15
	1 hour in the acid and 1 after water replaces the acid	81	12	7
Acetic acid 0·03 Mol. pH 3·5	1 hour	27	6	67
	1 hour in the acid and 1 after water replaces the acid	37	8	55

On can see, that in 100 % of the cells, maintained for one hour in CO_2, the movement begins again. In acetic and valerianic acid this movement does not reappear.

II. EXPERIMENTS WITH PARAMAECIUM CAUDATUM

The experiments were made with *Paramaecium caudatum*, cultivated in an infusion of hay in tap water. The infusoria were vitally stained by adding a solution of methyl red, concentrated by evaporation, to the liquid medium containing them [on the use of vital staining for determining the intracellular pH see PFEIFFER, 1927 (40) and SMALL, 1929 (49)].

This indicator turned out to be the only[1] one staining the cell rapidly and intensely, especially in conditions of acidification [see SCHAEDE, 1924 (46)]. It is well adapted in its range for experiments with CO_2 and has been used in similar researches by CHASE and GLASER (10), and by CHAMBERS (8). CHAMBERS pointed out the superiority of this indicator to neutral red, since the latter stains

[1] Except neutral red, which cannot be used because its range is unsuitable.

only the cell inclusions, whereas methyl red stains the protoplasm as well. However, it appeared that this stain also has its defects, namely, it changes very easily in an acidified medium, causing (especially with mineral acids) a strong displacement of pH. Therefore all the tests with acids, parallel to those with CO_2, had to be made with the mixture investigated placed in closed glass chambers, so as to prevent its coming into contact with atmospheric oxygen. Except this, all the tests of the pH of the medium had to be made exclusively by colorimetry, according to a buffer scale prepared by means of the same indicator; for if chinhydron was added to an acidified solution of the indicator, a reaction of some kind also took place, but one accompanied by an acidification. For the experiments solutions of seven different acids (HCl, H_2CO_3, H_3PO_4, oxalic, citric, acetic and valerianic acids) were used; they were added to the culture medium containing the infusoria, with the same quantity of indicator in each case, added in such proportion that pH of the acid solution should be the same as in the culture medium saturated with CO_2. For this purpose the pH of the mixture, acidified by the acid under investigation, was brought (according to the tint of the indicator) to the same pH as that determined for the culture medium saturated with CO_2 in a standard test tube, pH being measured with a precision of 0·05. The pH of the culture medium saturated with CO_2 was not always the same, varying from 5·0 to 5·3, according to the age of the culture, and to other unexplained factors. Because of this fact, and the consideration that the sensitivity of infusoria varies considerably with the age of the culture [see analogous indications of LOSINA-LOSINSKY, 1926 (33), JACOBS (23) and MALM (34)], the experiments with CO_2 and the acids were carried out at the same time, so that a control experiment with CO_2 should correspond to every experiment with an acid. The tests with the acids, as already said, were made in a glass chamber, having two glass inlet and outlet tubes furnished with rubber tubes and screw clamps. The mixture prepared was introduced into the chamber by aspiration, filling it entirely, and the clamps were then tightly closed. In such a chamber pH remained unchanged for 2—3 hours. For the experiments with CO_2 in a chamber like the one employed by LOPRIORE (31), through which CO_2 was made to pass, a hanging drop was made. The drop was stained by the indicator in the same proportion as in the experiment with acid. The pH within the cell was determined colorimetrically, by means of the ocular micro-scale prepared with leaflets from the Foliencolorimeter of WULFF, coloured by methyl red, like the scale coloured by anthocyanin in the experiment with *Tradescantia*, except that in the case of methyl red the scale was not enclosed in canada balsam, air having free access to it. Observations were also made, at fixed intervals of time, on the number of motile infusoria (expressed in percents) and of those whose movement had ceased. The cessation of movement coincided usually with the moment of death, as in *Paramaecium caudatum* there is no narcotisation period [see also JACOBS (23), NIKITINSKY and MUDREZOWA-WYSS (36)]. The results of the experiments on the action of CO_2 and of other acids upon *Paramaecium caudatum*, obtained by calculating the percentage of motile cells every 5—10 minutes, is given in table IV and in the curves (fig. 1—2).

According to their toxic action these acids in this case, as well, as in the experiment with *Tradescantia* can be divided into two sharply distinct groups. The first group includes all the mineral acids (HCl, H_2SO_4 and H_3PO_4) and, among organic acids, citric and oxalic acids, which are much less toxic than CO_2. This can be seen on the curve fig.1—2 (where time is marked on the abscissae and the percentage of motile *infusoria* on the ordinates), which go much higher than the curve corresponding to the experiments with CO_2. Also, this maximal survival of individual *infusoria* in acids of this group, is about twice as great as in CO_2. The coefficient of maximal survival K, obtained by dividing the time of complete dying off of the *infusoria* in the acid, by that of the mortality in CO_2, is here about two [from 1·8 (oxalic acid) to 2·5 (phosphoric acid)]. The second group includes valerianic and acetic acids, the toxicity of which is stronger than that of CO_2, as can be seen by the curve, which pass below the curve of CO_2, and by the factor K = 0·55—0·6 (i.e. in them the maximal

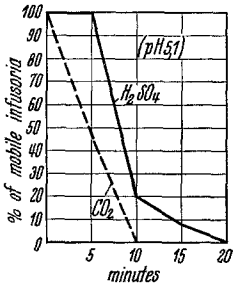

Fig. 1. Experiment 1. Curves showing toxicity of CO_2 and H_2SO_4.

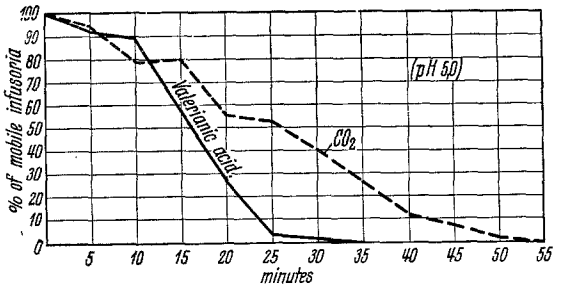

Fig. 2. Experiment 7. Curves showing toxicity of CO_2 and valerianic acid.

survival is half that in CO_2). Together with these counts, observations were made of the changes of colouring and of the state of the *infusoria*-cell. In the experiments of CHAMBERS (8) on *Amoeba dubia*, when CO_2 was made to pass through, only the cell inclusions were stained red, whilst the protoplasm itself remained of a yellowish colour. The same was observed with *Paramaecium*. In the latter case when CO_2 was passed through, the nutritive vacuoles and the granules in the endoplasm, whose pH was than according to the coloring about 4·9, were stained first of all, the protoplasm remaining yellow (pH 6·0). Evidently, here, as in the experiments of CHAMBERS, CO_2, when penetrating into the cell, was unable to acidify the strongly buffered protoplasm, and could only acidify the cell inclusions. Later, when the *infusoria* inflated and became rounded [JACOBS (23)], their movements became slower, the vacuoles were resorbed, and then a diffused colouration of the protoplasm appeared, showing a pH about 5·5, i.e. almost the same as that of the medium. The diffused colouring of the cellprotoplasm only takes place when death is imminent [see LOSINA-LOSINSKY (33), RUMJANTZEV and KEDROVSKY (45)], and the pH of the protoplasm is on a level with the pH of the medium [see CHAMBERS (8), REZNIKOFF and POLLACK (44) and POLLACK (41)]. After death, the *infusoria* became of a dark

Table IV

Experiments on the toxic action of CO_2 and other acids on Paramaecium caudatum, pH of the medium being the same, as well as the rapidity of penetration. (Number, in % of mobile individuals in the total number of infusoria).

$$k = \frac{\textit{moment of complete dying off in the acid}}{\textit{moment of complete dying off in } CO_2}$$

	pH of medium	k		Time in minutes																		
				5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Experiment 1	5·1	2	medium saturated by CO_2	44	0																		
			H_2SO_4 0·0027 Mol.	100	19	7	0																
Experiment 2	5·05	2·5	medium saturated by CO_2	44	15	0																	
			HCl 0.0032 Mol.	100	46	—	4	0															
Experiment 3	5·1	2·2	medium saturated by CO_2	79	48	25	19	12	0														
			H_2SO_4 0.0011 Mol.	100	85	67	65	—	30	—	—	7	—	—	1	0							
Experiment 4	5·1	1·8	medium saturated by CO_2	100	37	11	4	0															
			oxalic acid 0.0025 Mol.	88	79	—	60	—	5	—	1	0											
Experiment 5	5·2	2	medium saturated by CO_2	100	94	82	69	—	16	—	1	0											
			citric acid 0.0016 Mol.	100	93	—	91	—	72	—	52	—	25	—	17	—	11	—	2	—	3	—	0
Experiment 6	5·0	0·55	medium saturated by CO_2	94	79	80	55	52	40	—	12	—	1	0									
			CH_3COOH 0·0071 Mol.	92	89	75	38	9	0														
Experiment 7	5·0	0·6	medium saturated by CO_2	94	79	80	55	52	40	—	12	—	1	0									
			valerianic acid 0.0055 Mol.	93	89	57	26	2	1	0													

colour and the dye could be washed out of the cell. The reddening of the vacuoles was observed to occur at the same time as saturation with CO_2. The same was seen with all the other acids. Every where, within the limits of pH, the medium being 5·0—5·5, where the changes of colouring can be noticed, a reddening of the vacuoles, and consequently a penetration of the acid into the cell, was noticed instantly, as soon as the medium was acidified. But a diffused colouring, indicating that the cell was about to die, appeared earlier than in CO_2 in the case of acids with a more powerful action (valerianic and acetic acids), and later with acids of the first group, this being closely connected with the toxic action of these acids within the cell. Such external alterations as the swelling of the cells and the slowing down of the movements take place in the same manner in all the acids, excepting acetic acid, where a swelling of one extremity of the cell can frequently be observed, a transparent vesicle being formed, and the pellicule finally bursting.

Three conclusions can be drawn from the experiments with *Paramaecium*: 1. The penetration into the cell of all the acids investigated is the same for all, in the given conditions, coinciding with the moment of the acidification of the medium; 2. within the cell, these acids behave differently. Some of them show themselves more toxic (acetic and valerianic acid) and others less so (H_2SO_4, HCl, H_3PO_4, oxalic and citric acids) than CO_2; 3. This difference in their toxicity is nowise connected with the larger or smaller quantity of the H-ions connected with these acids, which penetrate into the cell; for when the colouring appears, the cell vacuoles present the same pH under the action of any one of the acids.

III. DISCUSSION OF THE RESULTS

As shown by an examination of data in the literature, the numerous researches dealing with the influence of CO_2 upon the living cell, have so far been carried on in two principal directions. On one hand, there has been the investigation of the specifity of CO_2 as compared to mineral acids (concerning its toxicity, as well as its narcotic influence), without entering into details of the mechanism of this phenomenon, and, on the other hand, a study of the rapidity of penetration of CO_2 into the cell, and of its acidifying action. As already said, in the endeavour to connect the questions of toxicity, narcotisation, rapidity of penetration of CO_2 and its acidifying effect, I had in mind, when beginning my work the following three questions. 1. Does the toxicity of CO_2 and other acids, as regards the cell, depend only on the different rapidity of their penetration? 2. Is the toxicity of CO_2 due only to the action of the H-ion, and hence is it necessary that there should be the same pH within the cell, in order to obtain an equal effect from the action of CO_2 and that of other acids? 3. Do the acids, which penetrate easily into the cell, exercise at the same time a narcotic action, or is this a specific property of CO_2? An answer to the first question can be found in the experiments with *Paramaecium*, in which we see, that with an equal permeability of the cell in the conditions of the experiment, with all the acids studied, a very different and higher toxic action is to be found in CO_2 than in the mineral, citric and oxalic acids, and a still stronger one in valerianic and acetic acids. In the case of *Tradescantia* this difference of toxicity is even more

marked, owing to the difference in permeability as regards different acids of the object studied (see 2-d series of experiments with *Tradescantia*). I am far from affirming absolutely, that all the acids penetrate into the cell with the same rapidity. The example of *Tradescantia*, where the penetration is by no means the same, is sufficient to prove that such is not the case. Certainly, the fact of a difference in penetration can be of great importance as regards the toxicity of acids, this circumstance being still more complicated, because permeability can apparently vary considerably according to the object observed, the quantity of acid, and the state of the cell [see GOMPEL (18)]; but, nevertheless, the difference of toxicity cannot be reduced solely to a difference in rapidity of penetration. An answer to the second question can be looked for, both in the first series of experiments with *Tradescantia*, and in those with *Paramaecium*. The first have shown that in order to obtain an equal effect (a cessation of movement in the protoplasm), very different concentrations of H-ions in the cells are required; lower ones for CO_2 and the acids of the second group, and higher ones for the acids of the first group. Likewise, it can be seen from the experiments with *Paramaecium*, that the acidification of the cell being equal, these can be considerable differences in the toxic effect, and therefore it does not depend solely on the H-ions concentration within the cell, but also, for CO_2 and the acids of the second group (as in the case of *Tradescantia*), on the concentration of the undissociated molecules of the acid, of which there will be a much greater number if the acid is a feebly dissociated one, than with strongly dissociated acids (pH being the same). The third series of experiments on *Tradescantia* gives an answer to the third question. There experiments have demonstrated, that in solutions of acetic and valerianic acids, no narcotic action manifests istelf, and that it does so only with carbonic acid. Evidently it is only the latter that can enter into an unstable reversible combination with the protoplasm, as pointed out by many of the above mentioned authors, whilst other acids, which also penetrate easily into the cell, produce an irreversible change in the protoplasm causing it to chie. On the grounds of the few observations, made so far the nature of the action, exercized by CO_2 upon the organism can be described thus, CO_2 has a strong capacity of penetration into the cell, and in this respect it is the most active among the acids. Acetic and valerianic acids, both feebly dissociated, are nearer to it than any others. In small doses it can act more like an acidifying agent, playing an important role in the physiological processes going on in the cell. In greater doses carbonic acid causes narcosis, and this property gives it a place apart from all other acids which penetrate easily into the cell, like acetic and valerianic acid. This action is no longer due to the concentration of the H-ion, but to the action of the undissociated molecule or probably of the anion. The fact of the existence of a narcotic dose is the most characteristic one for this acid. Still higher doses of acid are already lethal ones.

SUMMARY

Experiments on the comparative influence of CO_2 and other acids upon the living cell, carried out with two objects (*Paramaecium caudatum* and the hairs of the anther filaments of *Tradescantia virginica*) have shown the following:

1. The different toxicity of CO_2 and of other acids does not only depend on the different rapidity of their penetration (which in a number of cases is considerably higher than in other acids), for with an equally rapid penetration into the cell, the toxicity of these acids can be different.

2. According to the degree of their toxicity the investigated acids can be divided into two groups: I - including the mineral acids, citric acid and oxalic acid; and II - to which belong CO_2 and the acids near to it, easily penetrating into the cell, valerianic and acetic acids, which are more toxic, although their pH is the same as in the I-st group.

3. The toxicity of the second group of acids does not depend on their pH, but on the concentration of the undissociated molecules, as their effect is the same with a considerably less acid pH, than that of the acids of the first group.

4. Carbonic acid stands by itself among all these acids, because of its narcotic action, which apparently depends on the CO_2 molecule. The other acids are not able to cause narcosis.

5. Taking all this into consideration, carbonic acid can be characterised as an acid penetrating more easily into the cell than the other acids, acting as an acidifier in small doses, as a lethal agent in higher ones. Its penetrative and lethal properties do not depend on the H-ion concentration, but on the CO_2 molecule. The narcotizing capacity is particularly characteristic for CO_2 and creates a sharp difference between it and other acids.

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