ACID PENETRATION INTO LIVING TISSUES.

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The factors governing acid penetration into living tissues have never been clearly stated and we are therefore in ignorance as to the underlying physicochemical basis of the sour taste and allied phenomena. In this paper the writer presents quantitative evidence which he believes, shows: first, that acid penetration through a membrane occurs either in the form of the undissociated molecule or by the simultaneous passage of H^+ ion and anion; secondly, that the sourest acids are those which penetrate most rapidly; and finally that the relative rates of penetration of a series of acids are determined by the well known physicochemical laws of adsorption.

It was early realized that solutions of equal stoichiometrical acid concentration were not equally sour. Thus, for example, the threshold concentrations of HCl and CH_3COOH which just excite the sour taste are 0.001 N and 0.003 N respectively. Becker and Herzog (1907) state that the intensity of the taste sensation occasioned by acids of equal normality decreases in the following order: hydrochloric, nitric, trichloroacetic, formic, lactic, acetic and butyric. Paul (1922) in a series of papers on the physical chemistry of foodstuffs has determined the concentrations of acids which are equally sour with certain standard HCl solutions. Taking 0.005 N HCl as a reference he finds the order of increasing acid concentration is as follows: tartaric acid (lowest concentration therefore most sour), hydrochloric, acetyl lactic, lactic, acetic, carbonic. It is noteworthy that the two acids:—tartaric and acetyl lactic, which have practically identical dissociation constants (9.7×10^{-4}) are equally sour only when the former is 0.004 N and the latter 0.0059 N. Data such as this show that some other factor beside acid concentration plays a part in determining sourness intensity.

That this factor is not purely hydrogen ion concentration (or activity) must be evident from the data which I have obtained from experiments carried out in this laboratory. My experimental procedure consisted at first in giving 5 cc. samples of a number of concentrations of formic acid in the vicinity of the threshold concentration to fifteen people and thus determining the minimum concentration which was definitely sour. Astringent sensations are detectible in weaker solutions. A 5 cc. sample placed in the mouth is better than a few drops, as used by some investigators, because the former represents more nearly the average condition in the mouth rather than that of a single spot on the tongue. The mouth was rinsed with boiled distilled water between each test. Having determined the sour threshold concentration for formic acid, solutions of other acids were taken and that concentration of each acid chosen which was indistinguishable from the standard formic acid solution. On the average ten people passed judgement on each of these solutions. In cases where the acid had an odor the nose was held during each test. This effectively excluded the odor and restricted the judgment purely to the question of sourness. All solutions were at room temperature (22°–25°) and had been prepared from stock acid solutions of known concentration.

The hydrogen ion activities of these solutions were determined electrochemically by means of cells of the type Hg | HgCl, KCl saturated | acid | H_2, measurements being made at 20°C. Assuming that in these dilute solutions the activity coefficient of the H^+ ion is unity, we may write "concentration" instead of "activity." The data are recorded in Table I.

Since the values in Column 2 of this table differ from acid to acid it is evident that sourness is not purely a function of the stoichiometrical acid concentration. Further, since the hydrogen ion concentrations are not the same for the several acids we may conclude that this is not the determining factor. What then is the explanation of these results?

It occurred to the writer to compute the ratio of the concentrations of the undissociated part of each acid outside and inside the taste bud. The former concentration can readily be calculated from the data above, and are recorded in Column 2 of Table II. In the case of the

dibasic acids having anions $A^=$ and HA^- the concentration of the undissociated acid "outside" (H_2A) has been computed from the expression: Total acid concentration in mols $= H_2A\left(1 + \frac{K_1}{H^+} + \frac{K_1K_2}{(H^+)^2}\right)$

TABLE I.

Threshold Concentrations for Sour Taste.

	Total acid concentration	H^+ ion concentration
Formic	0.0018 N ± 3 per cent	0.00055
Acetic	0.0028 N ± 6 " "	0.00028
Butyric	0.0035 N ± 6 " "	0.00027
Valeric	0.0037 N ± 5 " "	0.00015
Oxalic	0.0020 N ± 8 " "	0.00116
Succinic	0.0032 N ± 7 " "	0.00034
Glutaric	0.0045 N ± 5 " "	0.00034
Lactic	0.0028 N ± 5 " "	0.00177
Tartaric	0.0022 N ± 7 " "	0.00070

TABLE II.

Concentration Gradients of Equally Sour Acids (from Threshold Concentration).

Acid	Concentration of undissociated acid Outside (*a*)	Relative concentration of undissociated acid Inside (*b*)	Ratio *a*/*b* when formic = 1
Formic	.00125	1	1
Acetic	.00252	11.8	0.17
Butyric	.00323	14.75	0.17
Valeric	.00355	13.4	0.21
Lactic	.00103	1.53	0.54
Oxalic	.0000285	0.0036	6.3
Succinic	.00134	3.2	.34
Glutaric	.00196	4.3	.38
Tartaric	.000444	0.22	1.60

where K_1 and K_2 are the first and second dissociation constants respectively. The inside concentration can be calculated, relatively but not absolutely, from the dissociation constants of the acids if we assume that the production of a given degree of sourness is due to the establishment within the cells of the taste bud of a definite hydrogen

ion concentration. Thus, for example, if two solutions of acetic acid and formic acid have the same hydrogen ion concentration; the concentrations of the undissociated parts stand together in the ratio $\frac{(CH_3COOH)}{(HCOOH)} = \frac{K_{formic}}{K_{acetic}} = \frac{2.14 \times 10^{-4}}{1.8 \times 10^{-5}} = 11.8$. In the case of dibasic acids the first dissociation constant K_1 was used for this computation and the effect of K_2 neglected, since it is impossible with the data available to take its effect into account quantitatively. The error will, however, be fairly small. These values are listed in Column 3 of

TABLE III.

Concentration Gradients of Equally Sour Acids, from Data by Paul and Bohnen.

Acid	Total acid concentration	H^+ ion concentration	Concentration of undissociated acid Outside (*a*)	Relative concentration of undissociated acid Inside (*b*)	Ratio *a*/*b* when formic = 1
	$\times 10^{-4}$ M	$\times 10^{-4}$ M	$\times 10^{-4}$ M		
Formic	7	3	4.0	1.00	1
Acetic	22	1.9	20.1	11.8	0.43
Propionic	33	2.56	30.4	14.75	0.53
Butyric	5	0.96	4.0	14.75	0.068
Monochloroacetic	12	8.0	4.0	0.138	7.3
Monobromoacetic	11	7.2	3.8	0.155	6.1
Lactic	23	5.0	18	1.55	2.9
Succinic	8	2	6	3.2	0.48
Malic	8	4	4	.53	1.9
Tartaric	4	3	1	0.22	1.1
Carbonic	53	0.4	52.6	705.	0.019

Table II under the heading "Relative concentration of undissociated acid inside." The ratios of the numbers in Columns 2 and 3 are found in Column 4, and these ratios may be taken to represent the relative gradients or driving forces which are necessary to cause the several acids to penetrate the tissues to a comparable degree (*i.e.* producing equal H^+ ion concentration inside).

Paul and Bohnen (see Paul, 1922) have obtained limiting concentrations for various acids which taste sour. Their results are given in Table III, Columns 2 and 3. I have calculated the quantities in the other columns from their experimental data.

Before discussing in detail the physicochemical basis for the different behavior of these acids in regard to taste excitation, I wish to draw attention to some experiments on the *rate* of acid penetration. Studies on rates have been made by Haas, Jacobs, Collett, Brooks, Crozier, Osterhout, Gompel and others. Crozier (1916) has measured the rate of penetration of various acids into the mantle fold of *Chromodoris zebra*. This tissue contains a natural indicator which changes color

TABLE IV.

Concentration Gradients of Acids, Penetrating Chromodoris Tissue and Producing a pH = 5.6 in 20 Minutes.

Acid	Total acid concentration	H^+ ion concentration	Concentration of undissociated acid Outside (*a*)	Concentration of undissociated acid Inside (*b*)	Ratio *a/b* when formic = 1
	$\times 10^{-4}M$	$\times 10^{-4}M$	$\times 10^{-4}M$	$\times 10^{-3}M$	
Formic	33.3	7.5	25.8	2.95	1.00
Acetic	188.8	5.8	183	34.7	0.60
Propionic	142.5	4.5	138	43.5	0.36
Butyric	93.4	3.6	89.8	43.5	0.24
iso-Valeric	38.4	2.4	36	39.4	0.10
Lactic	52.1	7.9	44.2	4.51	1.12
Benzoic	53.1	5.3	47.8	10.5	.52
Salicylic	35.1	14.4	20.7	.63	3.76
Succinic	84.7	7.1	77.6	9.56	0.93
Malic	74.0	15.3	58.7	1.58	4.26
Tartaric	66.6	21.0	45.6	.65	8.00
Oxalic	20.0	19.05	0.95	0.016	6.5
Malonic	52.6	22.1	30.5	0.39	8.8
Monochloroacetic	39.2	18.1	21.1	.41	5.95

from blue to pink at a pH of 5.6. Crozier's observations cover several concentrations of a dozen or more acids. I have replotted his curves of penetration time versus normal dilution and have determined graphically the concentrations of the various acids necessary to penetrate the tissue and produce the color change in a given time, for example, 20 minutes. Table IV shows these concentrations along with calculated values of the equilibrium concentration of the undissociated acids outside the tissue (*a*), and inside the tissue (*b*) when

pH = 5.6. The last column of the table gives the ratio a/b, which is a measure of the gradient driving the acid through the cell membrane.

The data in this table are for a penetration time of 20 minutes. If 30 minutes or 40 minutes had been chosen the a/b ratios would of course be changed but the general results would be the same. That is, the same trend would appear for the fatty acids, and the hydroxy acids lactic and salicylic would require a larger concentration gradient (a/b) than the corresponding acids, propionic and benzoic respectively. This is evident from the plot of penetration time versus normal dilu-

TABLE V.

Concentration Gradients of Acids Causing Retraction of Earthworm in 10 Seconds.

Acid	Total acid concentration	H^+ ion concentration	Concentration of undissociated acid Outside (a)	Relative concentration of undissociated acid Inside (b)	Ratio a/b when formic = 1
	$\times 10^{-3}M$	$\times 10^{-4}M$	$\times 10^{-4}M$		
Formic	11	14.3	96	1.00	1.00
Acetic	32	7.6	312	11.8	.28
Propionic	22.5	5.6	219	14.8	.15
Butyric	23	5.7	224	14.8	.16
iso-Valeric	18	5.3	175	13.4	.14
Caproic	11	3.9	107	14.8	.075
Caprylic	1	1.1	9	14.8	.006
Monochloroacetic	8.4	29.1	55	.138	4.17
Dichloroacetic	5.8	52.5	5.5	.0042	13.5

tion because there is no crossing of corresponding curves in the plot in this time interval.

Crozier (1917–18) has also studied the phenomena of sensory activation by acids in the earthworm *Allolobophora sp.* His method was to place an earthworm with one end in distilled water and the other in an acid solution and to measure the time of retraction of the worm from the acid solution. Again I have plotted his data and determined graphically the effective acid concentrations at fixed times, 10 seconds and 20 seconds, in which period the experimental accuracy seems to be greatest. Tables V and VI contain this material together with appropriate calculations by the writer. The gradients obtained from

Tables II to VI inclusive are collected in Table VII which allows a comparison to be made of the results of the different experiments. These are not absolute gradients but are relative, taking that for formic acid equal to unity. It should be quite evident that those acids which require the smallest gradient are those which penetrate the cell walls of the living tissue most easily.

In comparing the various sets of data attention should be focussed on the trends in the values for a series of acids rather than on the quantitative magnitude of the values for a given acid in the different experiments.

TABLE VI.

Concentration Gradients of Acids Causing Retraction of Earthworm in 20 Seconds.

Acid	Total acid concentration	H^+ ion concentration	Concentration of undissociated acid Outside (*a*)	Relative concentration of undissociated acid Inside (*b*)	Ratio *a/b* when formic = 1
	$\times 10^{-3}M$	$\times 10^{-4}M$	$\times 10^{-4}M$		
Formic	6	10.3	49.7	1.00	1.00
Acetic	15	5.1	145	11.8	25
Propionic	11	3.9	107	14.8	.14
Butyric	9.5	3.6	91.4	14.8	.12
iso-Valeric	7	3.3	66.7	13.4	.10
Caproic	4.5	2.5	42.5	14.8	.058
Caprylic	0.8	1.0	7.0	14.8	.0094
Monochloroacetic	6.2	24.2	38	.133	5.5
Dichloroacetic	4.7	43.3	3.7	.0042	17.6

Evidence for Adsorption by Cell Wall.—The fatty acid series shows a well marked trend from formic to caproic, particularly in the experiments on penetration rate, but less clearly in the taste data where figures are available. It seems likely that the higher members of the series are more readily adsorbed by the tissues than are the lower members. The last column of Table VII contains Freundlich's results on the relative concentrations of these undissociated acids which are necessary in order that charcoal may adsorb the same quantity of acid in each case. Thus for example if a butyric acid solution is 6 per cent as concentrated as a formic acid solution, the same quantity

of either acid will be adsorbed by charcoal. The excellent agreement between these results and those on the living tissues suggests that the fatty acids are taken into such tissues by an *adsorption process*. These acids show the same behavior in their toxic action on Ciliate Infusoria. In a paper on this subject Collett (1919) has measured the

TABLE VII.

Relative Concentration Gradients of Undissociated Acids across Living Tissues under Comparable Sets of Conditions.

Acids	From taste data		From penetration rate (Crozier)			From equal charcoal adsorption
	Taylor	Paul and Bohnen (see Paul, 1922)	*Chromodoris*	*Allolobophora*		
			20 min.	*10 seconds*	*20 seconds*	
Formic	1.00	1.00	1.00	1.00	1.00	1.00
Acetic	0.17	0.43	0.60	0.28	0.25	.68
Propionic		0.53	0.36	0.16	0.14	.22
Butyric	0.17	0.068	0.24	0.16	0.12	.06
iso-Valeric	0.21		0.10	0.14	0.10	
Caprylic				0.075	0.058	
Caproic				0.006	0.009	
Bromoacetic		6.1				
Chloroacetic		7.5	5.95	4.17	5.5	
Dichloroacetic				13.5	17.6	
Lactic	0.54	2.9	1.12			
Benzoic			0.52			
Salicylic			3.76			
Succinic	0.34	0.48	0.93			
Malic		1.9	4.26			
Tartaric	1.60	1.1	8.00			
Oxalic	6.3		6.5			
Malonic			8.8			
Glutaric	0.38					
Carbonic		0.019				

time necessary for a series of acids of equal pH to stop the beating of one-half the cilia taken for the test. He found the order of decreasing toxicity to be for paramecium at pH 3.5; valeric, butyric, propionic, acetic, formic. The same order held for *Euplotes* at pH 3.5 and pH 4.0. These results entirely oppose the old-fashioned idea that the cell walls have little holes through which an acid molecule may

squeeze for we actually find that the largest molecules, caprylic and caproic, enter most easily and the smallest (*i.e.* formic) enters with the greatest difficulty.

Influence of Polar Groups.—The retarding effect of polar groups is evident. On the average the necessary concentration gradients are approximately 5 times greater for the hydroxy acid than for the simple acid. Compare lactic and propionic, salicylic and benzoic, tartaric, malic and succinic. A halogen atom seems to have even greater effect. Thus the average ratio of chloroacetic to acetic is about 15, and two chlorine atoms added to acetic acid produce more marked retardation than two hydroxy groups added to succinic. Bromoacetic is like chloroacetic. It is noteworthy that carbonic acid, which may be looked upon as hydroxy-formic, does not have a gradient 5 times greater than formic but on the contrary it is 50 times smaller. It would appear that anhydrous CO_2 entered the tissues rather than H_2CO_3. Carbon dioxide is very soluble in oily liquids. That this gas has a very high penetration velocity was shown by Jacobs (1920), by measuring the time necessary for acid solutions of equal pH to produce a blue to pink color change in the natural indicator in the flowers of *Symphytum peregrinum.* Carbonic acid produced a color change in about 2 minutes, benzoic and valeric require about 15 to 30 minutes, and butyric, acetic and salicylic take longer times in the order listed. Jacobs stressed the relation between speed of penetration and sourness in the case of CO_2. It would seem as if the specific action of CO_2 on the respiratory center is largely due to its rapid penetration of the tissues even though other weak acids may be present. It is to be noted that Jacobs found salicylic to be slower than benzoic which agrees with Crozier's results on *Chromodoris.* It is difficult to estimate accurately the concentrations of equally sour benzoic and salicylic acids because of the fact that the latter has a very well marked *sweet* taste. This very fact is an indication of low cell membrane permeability because it is well known that other sweet tasting substances, sugars and polyhydric alcohols (which contain many hydroxyl groups) can penetrate cell membranes only with great difficulty. Gallic acid (trihydroxy benzoic) also has its sour taste obscured by a sweet taste.

One may ask why the adsorbability of a substance determines its

rate of adsorption. Comparing formic and butyric acids we see from Freundlich's experiments on charcoal that the latter acid is attracted more strongly by the adsorbent, or in thermodynamic terms that the free energy decrease on adsorption is greater. If we make the reasonable assumption that the adsorption mechanism is the same for all members of a given homologous series it follows that the adsorption velocity will be greatest where the free energy decrease is greatest. *One would therefore expect to find that for a given penetration velocity successively smaller concentration gradients are necessary as one changes from formic acid to its higher homologs.*

The nature of the cell membrane probably determines the effects noted in this paper. It is well known that the fatty acids depress the surface tension of water in the order: valeric > butyric >propionic > acetic > formic. According to the Gibbs theorem this fact means that their tendency to leave the body of the aqueous solution and concentrate on the surface follows the same sequence—hence their relative tendency to leave aqueous solutions and penetrate an *oily membrane*. The polar hydroxyl group and the chlorine atom increase the water solubility of an organic acid, consequently such substitution products have less tendency to enter an oil film than would the simple acid. Finally, oxalic acid is much more water-soluble than butyric, an acid having about the same molecular weight. It even forms a stable hydrate $C_2H_2O_4 \cdot 2H_20$. It is therefore much less likely to leave the aqueous solution and enter the membrane if the latter were of an oily character. There is of course a great deal of independent evidence as to the fat or lipoid nature of cell membranes.

Stereochemical Effects.

Another factor of very great importance in connection with acid penetration of tissues is the optically active character of the acid. It is well known that a large proportion of the chemical constituents of living tissues is made up of asymmetrical material. Pasteur was the first to separate an equimolecular mixture of *d*-acid and *l*-acid by addition of an optically active base, for example, quinine. The solubilities and other properties of the compounds so formed differ much more from each other than if an inactive base had been used. Consequently, since much tissue material is asymmetric, *d*- and *l*-acids

should be quite different in their physiological action. The fungus *Penicillium glaucum* grows readily in fumaric acid but only very slowly in a maleic acid solution. The reverse occurs in the case of *Lupinus albus* L., discovered by Kahlenberg and True (1896). Winther (1895) reviewed the literature on the action of organisms and reported that *d*-tartaric, *l*-lactic, *l*-mandelic, *d*-glutaminic and *l*-ethoxysuccinic acids have been prepared by the action of *Penicillium glaucum* on solutions of the racemic acids, showing that these optical acids are taken into the tissues of the fungus and metabolized less readily than their optical isomers. Fumaric acid has a pure acid taste, maleic is in time biting and nauseous; citraconic acid is bitter, mesaconic acid is sour. Quantitative experiments like those of Crozier on penetration rate would be of great interest in this connection and would probably lead to a clearer understanding of the chemical as well as physical nature of cell membranes.

Penetration Mechanism.

The writer has stressed the relation between experiments on penetration *velocity* and degree of *sourness* of various acids. Why is it that the excitation of a taste sensation seems to depend on a velocity factor? Let us consider an electrical analogy. If a copper wire placed in a magnetic field is suddenly jerked out a current is developed in the wire for a short time and may be observed with a ballistic galvanometer. On the other hand if the wire is removed slowly from the field only a very feeble current is developed. Here the time factor plays the major rôle. It would seem that the nervous impulse sent to the brain which we interpret as a sour taste requires for its excitation a rapid change in H^+ ion concentration in the taste bud. For this reason it is unlikely that data applicable for testing any theory of the sour taste can be obtained from experiments which measure H^+ ion or acid concentrations at *equilibrium conditions*. As an illustration of this latter type of experiment we have the work of Smith (1925) on the action of acids on cell division. "In these experiments the eggs were left in the solutions for one and a half to several hours, the time necessary to attain equilibrium between cells and fluid cannot influence the results, and has only theoretical significance."

All the intracellular effects noted in this paper have been considered

to be due to H^+ ion. This does not mean that H^+ ion alone has penetrated the cell walls leaving the anion outside because such a process would immediately build up an opposing electrostatic potential. There are, however, two ways by which H^+ ion might enter the cell interior. The first of these is by the simultaneous exit of another positively charged ion such as K^+ ion or Na^+ ion, comparable to the well known Cl^- ion—HCO_3^- ion exchange between red blood corpuscles and plasma during the respiratory cycle. If this alone were the determining factor we should find no differences between the sourness of acids of equal pH. On the other hand we find great differences, and further there is much evidence that Na^+ ions and K^+ ions do not pass very freely through cell membranes. We may therefore exclude this process in most cases. The second process is that as the H^+ ions are adsorbed into the cell membrane the electric charge so developed would attract the anion of the acid into the membrane as well, and thus the pair of ions would gradually penetrate into the cell interior. Such an assumption would seem to be necessary in the case of hydrochloric acid, which is of course sour and is able to penetrate many living tissues, and yet is considered to be completely ionized in accordance with the modern theory of strong electrolytes. We cannot here postulate that the undissociated HCl molecule is the only molecular species capable of penetration, because there is practically no HCl as such present. In order to be perfectly consistent in our treatment of weak organic acids and completely ionized inorganic acids we must assume that the former may also penetrate the membrane by the simultaneous passage of H^+ ion and anion. However, this mode of passage cannot be distinguished, electrically or thermodynamically, from the passage of undissociated molecules, since an ion pair will be neutral and since a temporary association of H^+ ion and anion during the penetration may be looked upon as a state of molecule formation. For solutions of equal pH the concentration gradient necessary to establish a given pH in the cell interior in a given time will therefore be determined by the ability of the anion to penetrate the cell membrane. The effects of polar groups on this phenomenon and the behavior of an homologous series have already been noted. Osterhout and Dorcas (1925) have studied the factors governing the penetration of H_2S and CO_2 into *Valonia macrophysa*. Their results are not inconsistent with the mechanism postulated in this paper.

SUMMARY.

The threshold concentrations for sourness of nine acids have been determined with an accuracy of about 8 per cent, and the H^+ ion concentration of these acids measured.

Calculations have been made of the relative concentration gradients of the undissociated acid across the cell membrane for a series of acids having equal sourness and also for a series of acids having equal penetration velocity as determined from experiments by Crozier on *Chromodoris* and on *Allolobophora*. For solutions of equal pH a high degree of sourness has been found to be associated with a high penetration velocity of the undissociated acid or of the anion. A comparison of these gradients with the results of adsorption experiments on charcoal indicates that the acids are taken into the tissues by an adsorption process.

Polar groups such as OH and Cl and Br are found to have a very marked effect in reducing the ability of organic acids to penetrate living tissues.

The important rôle of optical activity of the acids in determining their physiological action has been noted.

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