However, there seems to be little possibility of making a quantitative explanation. Considering the possible influence of variables which have not been controlled, one may conclude that the observed influence of dielectric constant of solvent upon the rate of conversion of ammonium cyanate into urea is in better agreement with the theory than one could reasonably expect. Scatchard, for reasons similar to those mentioned in the above discussion, called attention to the approximate character of Equation (7).

Summary

1. The rate of conversion of ammonium cyanate into urea has been studied at 50° in water (D 69.85) and in mixtures of water with methyl, ethyl and isopropyl alcohols and with 1,4-dioxane at dielectric constants for the mixtures of 65, 60, 55, 50, 45 and 40.

2. The primary salt effect in each solvent mixture is in good agreement with that predicted by the Brönsted–Christiansen theory for a reaction between monovalent ions of opposite charge.

3. The differences between rate constants, at equal dielectric constants, in the various solvent mixtures are explained from a consideration of the salting out of the non-aqueous solvents.

4. The influence of dielectric constant of the solvent upon the rate constants is in better agreement with the Scatchard–Christiansen theory than one could reasonably expect.

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Some Relationships between Molecular Structure, pH and the Ability of Bacteria to Grow in Solutions of Salts of Organic Acids

BY WILLIAM F. BRUCE

Some species of bacteria can grow in a solution which contains as the only source of carbon and energy the salt of a pure organic acid dissolved in a solution of inorganic salts similar to Ringer's solution.[1] If an organism can grow in a solution containing a particular acid as the sole source of carbon, it must possess a mechanism for decomposing this acid. Therefore the acids which support growth are possible intermediates in the decomposition of other organic materials, such as carbohydrates, by the organism in question. In addition, the decomposition of the growth-supporting acids themselves can be followed by chemical study, and solutions of these acids may be used to advantage for the culture of bacteria.

Failure of an organism to grow in a solution containing a single organic acid allows several alternative conclusions. The organism may not be able to decompose the acid; the acid or its decomposition products may be toxic to the bacteria in the concentration used; the organism may not be able to use the decomposition products for growing; or the organism may require the presence of other substances for growing. Thus definite toxic action of α-hydroxyisobutyric acid has been encountered in this study. Benzoic salicylic acids are, in general, toxic to organisms and Quastel[2] has shown by using the methylene blue technique that such molecules as formic and propionic acids can be attacked by *B. coli*, but nevertheless do not support growth by the organism. Some bacteria require hemin,[3] and addition of yeast extract to synthetic media may enable organisms to grow in solutions which do not support growth in the absence of yeast extract. Grey[4] has reported that the presence of formates may enable an organism to grow at the expense of substances which do not support growth in the absence of formate. Therefore, although failure to grow is evidence that the substance is not an intermediate in decompositions by the organism, it is not proof.

Previous studies[1a,c,5,6] of the growth of bacteria

(1) (a) H. Braun and C. E. Cahn-Bronner, *Centr. Bakt.* **1**, *Abt., Orig.*, **86**, 196 (1921); (b) W. F. Bruce, *J. Biol. Chem.*, **107**, 119 (1934); (c) J. Butterworth and T. K. Walker, *Biochem. J.*, **23**, 926 (1929); (d) T. K. Walker, V. Subramaniam, H. B. Stent and J. Butterworth, *ibid.*, **25**, 129 (1931); (e) S. A. Koser, *J. Bact.*, **8**, 493 (1923).

(2) J. H. Quastel, *Biochem. J.*, **19**, 641 (1925).

(3) E. O. Jordan and I. S. Falk, "Newer Knowledge of Bacteriology and Immunology," Univ. of Chicago Press, Chicago, Ill., 1928, p. 656.

(4) E. C. Grey, *Proc. Roy. Soc.* (London), **96B**, 156 (1924).

(5) H. C. Brown, J. T. Duncan and T. A. Henry, *J. Hyg.*, **23**, 6 (1924).

(6) J. H. Quastel and M. D. Wetham, *Biochem. J.*, **19**, 645 (1925).

in solutions of organic acids have included only a scattered selection of a few acids or else a mixture of substances. The present work involves the systematic study of the growth of bacteria in many solutions containing single pure organic acids. As a result, some general relationships between molecular structure and ability to support growth have appeared. Growth was more abundant and rapid in the *cis* acids than in the corresponding stereoisomeric *trans* acids. The lower fatty acids with *even* numbers of carbon atoms supported more growth than those with *odd* numbers of carbon atoms; but the corresponding α-hydroxy and amino acids with *odd* numbers of carbon atoms supported far better growth than the *even*-numbered substituted acids. A phenyl group introduced into the molecule has invariably reduced the ability of the substance to support growth. A methyl group introduced into some molecules increased, and in others decreased, the ability to support growth. A number of saturated and the corresponding unsaturated acids have shown the unique ability to support growth of the organisms over exactly the same range of *p*H. In general, growth was uniformly absent below *p*H 4, but the limit of growth on the alkaline side was not determined, because above *p*H 9 atmospheric carbon dioxide becomes a disturbing factor.

The importance of knowing the *p*H growth range in designing experiments for the isolation of intermediate products of bacterial action has already been established:[7] if a substance is produced in the course of a bacterial decomposition, selection of a *p*H at which it is not further decomposed in the medium may be essential for actual isolation of the substance.

The choice of the bacteria used in this study was the result of preliminary experiments with a large number of organisms. Four and occasionally six different species of bacteria which can be arranged in a graded series, depending on the number of different acids which support growth of each organism, were used in the work reported here. As representatives of organisms which utilize only a few acids, pathogenic bacteria were particularly useful.

The ability of the organisms to grow was selected as a criterion because where growth occurs in the presence of only one organic substance there is no escape from the conclusion that the organism can decompose the acid into products which it can use in its metabolism. Moreover, growth is an important function of bacteria in their natural environment. Aside from the practical importance of developing synthetic media for the growth of organisms, the chemical factors which condition growth and the chemical processes which growing bacteria carry on can be studied directly only by the use of growing organisms. The many complicating factors which influence growth can to some extent be cancelled out by the use of a standardized procedure for comparing growth on different acids.

(7) (a) C. Neuberg and M. Kobel, *J. Bact.*, **28**, 461 (1934); (b) E. W. Stearn and A. E. Stearn, *ibid.*, **26**, 9 (1933); (c) C. F. Arzberger, W. H. Peterson and E. B. Fred, *J. Biol. Chem.*, **44**, 472 (1920); (d) J. H. Quastel, *Biochem. J.*, **18**, 371 (1924).

Methods

The chemically pure organic acids were dissolved in a modified Ringer's solution to give a 1% concentration of the organic acid. The solution was divided into 10-ml. portions which had been adjusted to *p*H values in the series 4.0 to 9.0, sterilized, and inoculated with a culture of an organism carried on synthetic medium. Growth was recorded at intervals, and the final *p*H determined.

1. Media

The preparation of the fumaric acid solution will illustrate the method used for preparing media. To 150 ml. of water were added in the following order: 2.5 g. of fumaric acid, 51 ml. of 1 *N* NaOH (the equivalent amount), 1.18 g. of NH_4Cl, 0.25 g. of $MgSO_4 \cdot 7H_2O$, 15 ml. of 1 *N* HCl, 2.0 g. of K_2HPO_4, 3 drops of 0.01% $FeCl_3 \cdot CaCl_2$, $\frac{1}{4}$ *N* HCl to give *p*H 6.0, and water to make 250 ml. The solution was brought to *p*H 4 before addition of phosphate in order to prevent precipitation of magnesium ammonium phosphate.

This solution was divided into six 40-ml. portions and each portion was adjusted to a *p*H value in the series of six, beginning with *p*H 4.0 and ending with *p*H 9.0. To the first portion was added 4 ml. of 1 *N* HCl, to the second 1.4 ml. of 1 *N* HCl, to the fourth 0.8 ml. of 1 *N* NaOH, to the fifth 1.4 ml. of 1 *N* NaOH and to the sixth 2.6 ml. of 1 *N* NaOH. Each of these solutions was then sterilized by filtration in 10-ml. quantities into four sterile test-tubes (20 by 150 mm.).

Each *p*H series from *p*H 4.0 to 9.0 was inoculated with one 2-mm. loop per tube of a 2–4 day culture of one of the organisms in synthetic medium, and the tubes were incubated at 37.5°. After sixteen hours the first examination was made and growth was recorded. Four subsequent examinations were made at twenty-four-hour intervals and the final *p*H in each tube was determined.

The important variables in the composition of the medium were the organic substrate and the *p*H. The inorganic constituents remained practically the same throughout. A slight variation in the amount of sodium present was due to a small variation in the number of milliequivalents of the different acids. Citric and acetic acids in 1% solution, for example, are, respectively, 0.0156 and 0.0166 *N*. Experiments with various concentrations

showed that within the limited range of equivalent concentration encountered here, the variations had no observable effect on the growth of the bacteria. Higher concentrations of the organic acids, for example 3% (0.047 *N*) citric acid, retarded the growth of the organisms.

The concentrations of inorganic salts which were used have been found satisfactory by other workers with other organisms.[1] The magnesium concentration varied regularly in each *p*H series because of the precipitation of magnesium ammonium phosphate at *p*H 8 and 9. While this can easily be avoided by reducing the amount of magnesium to a trace, greater concentrations were used when possible because they have been preferred by Braun and Cahn-Bronner,[1a] Koser[1c] and Quastel.[2] During the earlier experiments the precipitate was kept in the solution, but this proved to be unnecessary because when growth occurs the reaction nearly always becomes more alkaline and more of the precipitate is deposited. To avoid any danger of confusing the precipitate with growth, the solution was therefore filtered before sterilizing. Exceptions to the shift to an alkaline reaction when growth occurs were found in aconitic and tricarballylic acid solutions; *B. aertrycke* (smooth) in pyruvate medium changed the *p*H from 7 and 8 to 6.3 and 6.4, respectively.

The insolubility of some acids limited the number which could be studied. Those which proved relatively insoluble throughout the entire *p*H range were agaric, α-aminocaprylic, azelaic, cyclohexanecarboxylic, lauric, sebacic, stearic, and undecylenic acids and tyrosine. Uric and *p*-chlorobenzoic acids were very slightly soluble from *p*H 4 to 6, but were sufficiently soluble from *p*H 7 to 9. A solution saturated at 25° was used where these acids were soluble to the extent of less than 1%.

2. Sterilization

The solutions were for the most part sterilized by filtration, because many of the organic acids undergo some degree of decomposition when their solutions are autoclaved. This instability is particularly apparent in acetonedicarboxylic acid, which decomposes completely into acetone and carbon dioxide at 80°. Other substances which seem more stable to heat nevertheless show by the development of color, particularly in the alkaline region, that some chemical changes have occurred when their solutions are autoclaved. In order to avoid possible chemical contamination from the metal parts of the ordinary bacteriological filters, since traces of metals may have great influence upon growth of bacteria in synthetic media,[8] glass filters of the Seitz type were used for the sterilization.[9]

By using Gram-stained smears from the tubes in which growth occurred, contamination of less than 0.3% of the tubes by molds or other organisms was detected. The series in which contamination occurred were repeated without further difficulty. In fact most of the observations were checked by repeated experiments at different times and except for some cases in which very faint growth appeared were found to be reproducible within a *p*H unit of either end of the range. The solutions of the most stable substances, such as acetic, propionic and butyric acids, were autoclaved in one series of experiments and filtered in another. The growth range in the one set of solutions duplicated that in the other within a *p*H unit. The Gram-stained preparations from tubes in which growth occurred showed that all the tubes except those in which mold was found contained uniform Gram-negative organisms similar to the original culture, together with the faintly stained forms which occur in old cultures. Sterilization by filtration thus is shown to have been adequate for these solutions; the solutions, to be sure, contained few if any bacteria to begin with.

3. Organisms

The rough and smooth forms of *B. aertrycke* which were used have been previously described.[1b] Two additional strains of the smooth form freshly isolated from guinea pigs did not differ from the original smooth strain in their growth on citric acid. A fresh culture of *B. pyocyaneus* isolated from a bladder infection was secured from Dr. J. H. Brown, and carried on synthetic citrate medium at room temperature by weekly transfers. By microscopic examination and plating on nutrient agar, only short Gram-negative rods of a uniform colony type could be seen. A fresh culture of *B. bronchisepticus* was obtained from Dr. J. B. Nelson and since growth on citrate medium is slight, was at first carried in peptone water, later in maleic acid medium at 37°. It is composed of short, rounded Gram-negative rods. The culture of *B. paratyphosus* B is a laboratory strain secured from Dr. C. Ten Broeck. This organism behaved like the rough form of *B. aertrycke;* and *B. enteritidis*, a laboratory strain secured from Dr. Nelson, also showed little deviation from the behavior of the rough form of *B. aertrycke* (Table IV). Inoculation was made by a 2-mm. loop, and the dilution of the medium transferred with the organisms was 1:5500. The relation between the growth of the rough and smooth forms of *B. aertrycke* in these solutions is like that in the more complicated media of the bacteriologist. The rough form grows much more readily than the smooth.

4. pH and Growth

The *p*H of the solutions was determined colorimetrically both before and after incubation. Occasional concurrent determinations by means of the quinhydrone electrode showed that a variation of more than 0.1 *p*H unit from the value determined colorimetrically was rare. The solutions were sufficiently well buffered to prevent a shift of more than 0.5, or occasionally 1 *p*H unit, until the second observation for growth. Where abundant growth occurred, the solutions became more alkaline by as much as 3 *p*H units during the five days or more over which the observation extended. Numerous tests showed, however, that no change greater than 0.3 *p*H unit occurred in the solution during the first sixteen hours of growth. For the solutions in which abundant growth and a larger shift in *p*H were found at the end of the observation period, these first two observations had already shown the optimum *p*H and relative amounts of growth. For the slower growing organisms, the full five-day period or longer was necessary; but in these cases the amount of chemical change was seldom sufficient to cause a measurable shift in *p*H.

There was in general more growth at *p*H 7–8 than in any other region. *B. pyocyaneus* was particularly able to

(8) E. O. Jordan and I. S. Falk, *op. cit.*, p. 284.
(9) W. F. Bruce, *Science*, **81**, 179 (1935).

grow in the more alkaline solutions, in some cases producing a final *p*H of 9.5. The media at *p*H 9, except for instances in which very heavy growth appeared, absorbed enough carbon dioxide by the end of the observation period to bring the final *p*H to 8.5. In a few cases, namely, acetonedicarboxylic, *l*-ascorbic, phenyl-pyruvic and thioacetic acids, a shift of the *p*H without growth indicated spontaneous chemical change, accompanied in one case by a cloudy deposit, shown by subculturing not to be growth, and in another by the development of color.

A remarkable example of the intimate connection between *p*H and the ability of bacteria to grow in a given medium was found in mandelic acid. In the medium prepared from this acid only *B. pyocyaneus* grew, and that only at *p*H 8, not at *p*H 7 or 9. Growth appeared on the fourth day, when it was rapid and abundant. A second trial at another time exactly reproduced this result. In no other medium was the *p*H growth range found so narrow for an abundant growth.

5. Measurement of Growth

Incipient growth was detected by the presence of a Tyndall effect when a beam of light was passed through the tube held against a dark background. The initial medium was perfectly clear and gave no Tyndall effect, but faint growth was readily detected as an even opalescence. This could scarcely be confused with the more granular material deposited by the spontaneous decomposition of thioacetic acid or by the formation of magnesium ammonium phosphate, but in case of any doubt on this score, solution was subcultured to citrate or peptone medium in which the organisms are known to grow well. The formation of a thin surface film was sometimes the first evidence of growth. This type of growth then produced the opalescence shown by the Tyndall effect. In many tubes, however, growth was evidenced by more or less turbidity. The cultures were first examined after sixteen hours and then at twenty-four-hour intervals. In no case did growth in a *p*H series begin after five days.

For purposes of a rough quantitative comparison, the tubes in which growth was found were divided into three groups. The first group, designated (+) in the tables, comprised solutions in which faint growth occurred, the organisms being present in numbers sufficient to show unmistakable growth, absorbing or reflecting up to 10% of the light transmitted to a photronic cell by a tube (20 × 150 mm.) of clear medium. The second group (++) was composed of moderately turbid solutions which intercepted from 10 to 40% of the light, and the third (+++) of very turbid solutions intercepting from 40 to 80% of the light. Some flexibility in this classification was necessary because in some tubes pellicle formation resulted in uneven dispersion of the growth and hence misleading readings from the photronic cell, and in others the presence of magnesium ammonium phosphate occasionally interfered. Moreover, in order to show distinct differences between the tubes in a *p*H series it was occasionally necessary to rate a tube +(+) which did not intercept quite 10% of the light.

Experimental

1. Detailed Record of Growth in Fumaric Acid Medium

For each organic acid detailed observations of growth were made over a range of *p*H from 4 to 9, and over an interval of five days or more. As an example, Table I shows a detailed protocol of these observations for fumaric acid. The table shows that both *B. pyocyaneus* and *B. aertrycke* (rough) grew abundantly in fumaric acid medium at *p*H 6, 7 and 8 within sixteen hours. *B. pyocyaneus* in addition grew abundantly at *p*H 9. Both organisms grew poorly at *p*H 5, but as the *p*H shifted on account of growth, the organisms grew much more abundantly. Although *B. aertrycke* (rough) in one experiment grew at *p*H 5, in the majority of experiments it failed to grow at *p*H 5. *B. bronchisepticus* showed a delayed growth

TABLE I

DETAILED RECORD OF GROWTH IN FUMARIC ACID MEDIUM

	B. pyocyaneus Days						*B. aertrycke* (rough) Days					
*p*H initial	1	2	3	4	5	*p*H end	1	2	3	4	5	*p*H end
4.0	0	0	0	0	0	4.0	0	0	0	0	0	4.0
							(+)	(++)	(+++)	(+++)	(+++)	8.3
5.0	0	+	++	+++	+++	8.1	0	0	0	0	0	5.0
6.0	++	+++	+++	+++	+++	8.0	++	+++	+++	+++	+++	8.4
7.0	++	+++	+++	+++	+++	8.0	++	+++	+++	+++	+++	8.4
8.0	++	+++	+++	+++	+++	8.4	++	+++	+++	+++	+++	8.8
9.0	++	+++	+++	+++	+++	9.3	0	0	+	++	++	9.2

	B. bronchisepticus Days						*B. aertrycke* (smooth) Days					
*p*H initial	1	2	3	4	5	*p*H end	1	2	3	4	5	*p*H end
4.0	0	0	0	0	0	4.0	0	0	0	0	0	4.0
5.0	0	0	0	0	0	5.0	0	0	0	0	0	5.0
6.0	0	0	0	+	++	6.2	0	0	0	0	0	6.0
7.0	0	+	+	++	++	7.0	0	0	0	0	0	7.0
8.0	0	0	0	+	++	8.0	0	0	0	0	0	8.0
9.0	0	0	0	+	++	8.6	0	0	0	0	0	8.6

which eventually became fairly abundant, but not enough to cause an appreciable shift in *p*H. *B. aertrycke* (smooth) did not grow in fumaric acid medium at any *p*H. The table shows further that absorption of carbon dioxide was sufficient to cause a shift of *p*H from 9 to 8.6, except where vigorous growth increased the *p*H to 9.3.

2. Growth of Bacteria in Solutions of Cis and Trans Acids

Table II shows that in media prepared from the *cis* acids, citraconic and *cis*-aconitic, growth is more abundant than in the media prepared from the corresponding stereoisomeric *trans* acids, mesaconic and *trans*-aconitic. In maleic acid (*cis*) growth is nearly equal to that in fumaric acid (*trans*). This apparent exception to the more abundant growth on *cis* acids can be explained by the fact that fumaric acid may readily be converted into malic acid,[10] which supports excellent growth. A comparison of angelic and tiglic acids which has been made very recently shows that the *cis* acid (angelic) supports growth by *B. pyocyaneus* and *B. aertrycke* (rough), whereas the *trans* acid supports no growth.

TABLE II

THE GROWTH OF BACTERIA IN SOLUTIONS OF SALTS OF CIS AND TRANS STEREOISOMERIC ACIDS

Organisms: P = *B. pyocyaneus*, R = *B. aertrycke* (rough), T = *B. paratyphosus* B (rough), B = *B. bronchisepticus*, S = *B. aertrycke* (smooth). Underscoring indicates growth in 16 hrs. (*e. g.*, ±, ±± or ±±±) and the number of + signs indicates the extent of growth finally obtained.

Cis Series

Acid		*p*H 4	5	6	7	8	9
Maleic	P	0	0	+++	±±	±	0
	R	0	0	±±±	±±	±±	±
	T	0	0	++	+	+	0
	B	0	0	0	+	++	+
	S	0	0	0	0	0	0
Citraconic	P	0	0	0	±±	±±	+
	R	0	0	0	+	++	++
	B	0	0	0	+	±±	++
	S	0	0	0	0	0	0
Aconitic (*cis*)	P	0	±±±	±±±	±±±	±±±	++
	R	0	±±±	±±	±±	+	0
	T	0, +	±±±	±±±	±±±	±±	0
	B	0	0	±±	±±	+	0
	S	0	±±	±±±	++	+	0

Trans Series

Acid		*p*H 4	5	6	7	8	9
Fumaric	P	0	+	±±±	±±±	±±	±±
	R	0	±, 0	±±±	±±±	++	++
	B	0	0	++	±±	++	++
	S	0	0	0	0	0	0
Mesaconic	P	0	0	++	±±	±±	++
	R	0	0	0	0	0	0
	B	0	0	0	0	+	+
	S	0	0	0	0	0	0
Aconitic (*trans*)	P	0	+++	±±±	±±±	+	+
	R	0	±±±	±±	++	+	0
	B	0	0	+	±±	+	+
	S	0	0	0	0	0	0

(10) K. P. Jacobsohn and F. B. Pereira, *Bull. soc. chim. biol.*, **16**, 550 (1934).

3. Effect of the Number of Carbon Atoms on the Growth of Bacteria in Solutions of the Lower Fatty Acids

Table III shows that the organisms grow more readily in solutions of the unsubstituted lower fatty acids with *even* numbers of carbon atoms than in those with *odd* numbers of carbon atoms. With increasing molecular weight, growth decreases and above five carbon atoms no differences between the *even* and *odd* acids is apparent. Thus the *odd*-numbered unsubstituted fatty acids formic, propionic and propiolic, support little or no growth, and valeric with five carbons supports only moderate growth. Acetic, *n*-butyric, isobutyric and *n*-caproic, the *even*-numbered un-

TABLE III

EFFECT OF THE NUMBER OF CARBON ATOMS ON THE GROWTH OF BACTERIA IN SOLUTIONS OF THE LOWER FATTY ACIDS

Organisms: See Table II; E = *B. enteritidis*

A. Odd-Numbered Acids

Unsubstituted acids

Acid		*p*H 4	5	6	7	8	9
Formic	P	0	0	0	0	+	0
	R, B				no growth		
Propionic	P	0	0	0	0	+	0
	R	0	0	0	0	+	0
	B, S				no growth		
Propiolic[a]	P, R, B, S				no growth		
Valeric	P	0	0	0	0	++	±±
	R	0	0	0	0	+	+
	B	0	0	0	0	+	+(+)
	S	0	0	0	0	0	0

Hydroxy acids

d,l-Lactic	P	0	0	±±	±±±	±±±	±±±
	R	0	+	±±±	±±±	±±±	±±
	T	0	+	±±±	±±±	±±±	±±
	E	0	0	+	+++	0	0
	B	0	0	0	++	++	+
	S	0	0	+	+	0	0
d,l-Glyceric	P	0	±	±±	±±	±±	++
	R	0	±±±	±±±	+++	+++	++
	S	0	0	0	0	+	0
α-Hydroxy acrylic (pyruvic)	P	0	0	+++	±±±	±±±	±±±
	R	0	0	++	±±±	±±±	++
	B	0	0	0	+	+	+
	S	0	0	0	++	++	0

Amino acids

d,l-Alanine	P	0	+++	±±±	±±±	±±±	+++
	R	0	+++	±±±	±±±	±±	0
	B	0	0	+	++	±±	0
	S	0	0	0	0	0	0
d,l-α-Amino-*n*-valeric	P	0	+	++	0	0	0
	R, B, S				no growth		

TABLE III (*Concluded*)

B. Even-Numbered Acids

Unsubstituted acids

Acid		pH 4	5	6	7	8	9
Acetic	P	0	0	0	±±	++	+0
	R	0	0	0	++	++	0
	B	0	0	0	0	+0	+0
	S	0	0	0	0	0	0
n-Butyric	P	0	0	0	+	±±±	++
	R	0	0	0	0	+	+
	B	0	0	0	0	+0	0
	S	0	0	0	0	0	0
Isobutyric	P	0	0	0	0	+++	+++
	R	0	0	0	0	+(+)	+
	B, S				no growth		
n-Caproic	P	0	0	0	0	±±	±
	R, B, S				no growth		

Hydroxy acids

Glycolic	P	0	0	±	±	0	0
	R, B, S				no growth		
β-Hydroxy-*n*-butyri-[b]	P	0	0	0	+++	+++	+++
	R	0	0	0	+	+	+
	B	0	0	0	0	++	++
	S	0	0	0	0	0	0
α-Hydroxy isobutyric	P, R, B, S				no growth		

Amino acids

Glycine	P	0	0	+	++	+(+)	0
	R, B, S				no growth		
d,l,α-Amino-*n*-butyric	P, R, B, S				no growth		
d,l-α-Amino-*n*-caproic	P, R, B, S				no growth		

[a] Prepared by Dr. H. S. Rhinesmith.

[b] A commercial sample which proved impure was used at first. The sample in this report was secured from another source and purified by distillation *in vacuo*.

substituted acids, support moderate to good growth.[11]

On the other hand, the α-hydroxy and amino substituted acids with *odd* numbers of carbon atoms, namely, *d,l*-lactic, *d,l*-glyceric, α-hydroxyacrylic and *d,l*-alanine support excellent growth; whereas the *even*-numbered α-hydroxy and amino acids support little or no growth: glycolic, α-hydroxyisobutyric, glycine, *d,l*-α-amino-*n*-butyric, *d,l*-α-amino-*n*-caproic; β-hydroxy-*n*-butyric acid supports good growth.

The experiments of Koser[1e] show that *B. coli* grows much more readily in α-hydroxypropionic acid than in propionic acid medium, and that *B. coli aerogenes*, which does not grow on propionic acid, grows abundantly in α-hydroxypropionic acid media, which is in accord with the observations made in this study. The toxic action of α-hydroxyisobutyric acid was observed when 0.5 ml. of the medium containing 1% of this acid upon addition to 10 ml. of citrate medium prevented growth by *B. aertrycke;* 0.1 ml. did not prevent growth. The number of possible substituted 4 carbon acids is much greater than the number known or available. The rule concerning substituted acids can therefore not yet be regarded as general in application, although all the substances thus far studied are in agreement with it.

(11) For this work reagent grade acids were further purified by fractional distillation. The amount of inoculum was kept small and taken from synthetic media, for addition of 0.1 ml. of a 1:100 20% yeast infusion was sufficient to allow fairly good growth of *B. pyocyaneus* in 10 ml. of propionic acid medium.

4. Growth of Bacteria on Acids of Special Chemical Structure

Table IV shows that little or no growth occurs on compounds with stable ring structures, namely, barbituric, *d*-camphoric, furoic, and kojic acids. Some acids of ring structure, namely, *l*-quinic and uric, support moderate growth by the adaptable *B. pyocyaneus*. This shows that while bacteria can sometimes open such a ring, they do so with difficulty.

TABLE IV

THE GROWTH OF CERTAIN BACTERIA IN SOLUTIONS OF SALTS OF ORGANIC ACIDS OF SPECIAL CHEMICAL STRUCTURE

Organisms: P = *B. pyocyaneus*, R = *B. aertrycke* (rough), T = *B. paratyphosus* B (rough), E = *B. enteritidis*, B = *B. bronchisepticus*, S = *B. aertyrcke* (smooth). Underscoring indicates growth in 16 hrs.

Acid		pH 4	5	6	7	8	9
Acetone-dicarboxylic	P	0	++	±±±	±±±	++	0
	R	0	0	0	0+	0+	0
	B	0	0	+	+	+	0
	S	0	0	0	0	0	0
Aceturic	P	0	0	±±±	±±±	±±±	++
	R	0	0	±	±	0	0
	B	0	0	++	++	±	0
	S	0	0	0	0	0	0
Adipic	P	0	0	+	±±	+++	0
	R, S				no growth		
l-Ascorbic	P	0	0	±	±±	±±	0
	R	0	++	±±	±±	±±	++
	S	0	0	0	0	0	0
l-Asparagine	P	0	++	±±±	±±±	±±±	±±±
	R	0	+++	±±±	±±±	±±±	++
	B	0	0	0+	0+	0	0
	S	0	0+	0+	0+	0	0
Aspartic	P	0	+	±±±	±±±	±±±	+++
	R	0	0	++	±±±	++	+
	B, S				no growth		
Barbituric	P, R, S				no growth		
Benzoic	P, R, B, S				no growth		
d-Camphoric	P	0	+	+	0	0	0
	R	0	0	±	+	0	0
	B, S				no growth		
Chloroacetic	P	0	0	0	0	+	0
	R	0	+	+	+	0	0
	B, S				no growth		
o-Chloro-benzoic	P, R, B, S				no growth		
m-Chloro-benzoic	P, R, B, S				no growth		
p-Chloro-benzoic	P, R, B, S				no growth		

TABLE IV (*Concluded*)

Acid		*p*H 4	5	6	7	8	9
Cinnamic	P,	R, T, S			no growth		
Citric	P	0	+	±±±	±±±	±±±	±±±
	R	0	±±±	±±±	±±±	±±	±
	T	0	±±±	±±±	±±±	±±±	±±
	B	0	0	+	±±	±±	+
	S	0	+	±±	±±	+	0
α,α′-Dihydroxy adipic (*meso*)	P	0	±	±	±	±	0
	R,	B, S			no growth		
Furoic	P,	R, S			no growth		
Gallic	P,	R, B, S			no growth		
d-Glutamic	P	0	++	±±±	±±±	±±±	+
	R	0	++	±±±	±±±	±	0
	B	0	0	++	+++	0	0
	S	0	+	++	+++	0	0
Glutaric	P	0	0	±±	±±±	++	+
	R,	S			no growth		
α-Glycerophosphoric	P	0	++	±±±	±±±	++	0
	R	0	+++	±±±	±±±	±±±	0
	B	0	0	+	+	0	0
	S	0	0	0	0	0	0
Hippuric	P,	R, T			no growth		
m-Hydroxybenzoic	P	R, B, S			no growth		
p-Hydroxybenzoic	P	0	0	0	+++	+++	+++
	R,	T			no growth		
Isocitric	P	0	0	±±±	±±±	±±±	±±
	R	0	+++	±±±	±±±	±±	±
	B	0	+	±±±	±±±	±±	±
	S	0	+	±±	++	++	+
Itaconic	P	0	0	±±	±±±	±±±	++
	R	0	0	++	++	0	0
	T	0	0	+	+	0	0
	S	0	0	0	0	0	0
Kojic	P,	R, S			no growth		
Levulinic	P,	R, S			no growth		
l-Malic	P	0	0	++	±±	±±	+
	R	0	++	±±±	±±±	±±	±
	T	0	++	±±±	±±±	±	+0
	B	0	0	0	+	+	+
	S	0	+0	+	+	0	0
Malonic	P	0	0	±±±	±±±	±±	++
	R,	T, B, S			no growth		
Mandelic	P	0	0	0	0	+++	0
	R,	T, B, S			no growth		
Mucic	P	0	0	+++	+++	+++	0
	R	0	±±	±±±	±±±	±±	±±
	B	0	0	0	+	+	+
	S	0	0	0	0	0	0
Oxalic	P	0	0	±	±	±	0
	R,	B, S			no growth		
o-Phthalic	P	0	0	±	±	±	0
	R,	B, S			no growth		
Phenylparaconic	P	0	0	0	+	+(+)	0
	R,	B, S			no growth		
Phenylpyruvic	P	0	0	0	+	++	0
	R,	B, S			no growth		
Pyro-tartaric	P	0	±	±	±±	±±±	+++
	R	0	0	0	±	+	0
	B	0	0	0	+	±	+
	S	0	0	0	0	0	0
l-Quinic	P	0	0	±±	±±	±±	±±
	R,	S			no growth		
Salicylic	P,	R, B, S			no growth		
Succinic	P	0	0	±±±	±±±	±±±	±±±
	R	0	0	±±±	±±±	++	++
	B	0	0	±±	+	+	+
	S	0	0	0	0	0	0
Sulfanilic	P	0	0	0	+	+	0
	R	0	0	+	+	+	0
	B,	S			no growth		
d-Tartaric	P,	R, T, B, S			no growth		
Thioacetic	P,	R, S			no growth		
Tricarballylic	P	0	+++	±±±	±±±	++	++
	R	0	±±±	±±±	±±	±	+
	B	0	0	0	0	0	0
	S	0	0	+	0	0	0
Uric	P	0	+	±±	±±	+	0
	R,	B, S			no growth		

The presence of a phenyl group greatly decreases the ability of a substance to support growth. There is thus no growth in benzoic, *o*-, *m*- and *p*-chlorobenzoic, cinnamic, gallic, hippuric, *m*-hydroxybenzoic, *o*-phthalic, salicylic and sulfanilic acids. But some compounds containing phenyl groups support fairly good growth by the most adaptable organisms, *B. pyocyaneus*: *p*-hydroxybenzoic, phenylparaconic, phenylpyruvic and mandelic acids.

Introduction of a methyl group into the molecule may have either a favorable or an unfavorable effect on ability to support growth. Methyl glycolic (lactic) acid (Table III), for example, supports much better growth than glycolic acid. Acetic acid is more available than formic, and butyric than propionic acid. But methylsuccinic (pyrotartaric, Table IV) is less readily utilized than succinic acid, and the higher fatty acids, built up by the addition of methyl groups, support little or no growth. When a functional group such as carboxyl is methylated, the substance no longer supports growth. This is the fact for trimethyl citrate. It is in agreement with the experiments of Koser on methylated glucose[12] and with the statement of Quastel[13] that "any modification of the carboxyl groups which prevents ionization of the latter should diminish or entirely remove hydrogen donating power."

Table IV shows that a number of saturated acids are uniquely similar in *p*H growth range to the corresponding unsaturated acids. Thus (a) succinic and fumaric, (b) tricarballylic and aconitic, (c) pyrotartic, mesaconic and itaconic acids support growth within *p*H ranges practically identical within each group. This fact is evidence that the three forms under (c) are intimately related in the mode of their decomposition, and that the relation in all these groups is like that already well established for succinic and fumaric acids.[14]

(12) S. A. Koser and F. Saunders, *Proc. Soc. Expll. Biol. Med.*, **30**, 443 (1933).

(13) J. H. Quastel, *Biochem. J.*, **20**, 166 (1926).

(14) (a) J. Lehmann, *Skand. Arch. Physiol.*, **58**, **173** (1930); (b) H. Borsook and H. F. Schott, *J. Biol. Chem.*, **92**, 535 (1931); (c) J. Lehmann, *Skand. Arch. Physiol.*, **65**, 291 (1933).

Here the first step in the decomposition of the saturated acid is reversible dehydrogenation to the unsaturated acid, and the process occurs with surprising ease, in view of the stability of succinic acid to permanganate.

Table IV also contains substances which are possible intermediates in a variety of bacterial decompositions. A possible first stage in the decomposition of citric acid by bacteria is the formation of equimolecular amounts of acetonedicarboxylic and formic acids.[1c,5] Since acetonedicarboxylic acid supports good growth by *B. pyocyaneus* in the *p*H range in which citric acid also supports growth, the assumption that acetonedicarboxylic acid may be an intermediate is admissible for *B. pyocyaneus;* but since *B. aertrycke* fails to grow in acetonedicarboxylic acid medium, the presumption is that this acid is not an intermediate in the decomposition of citric acid by the latter organism. Acetonedicarboxylic acid has in fact been found in the decomposition of citric acid by *B. pyocyaneus*[1c] but could not be detected in the decomposition of citric acid by *B. aertrycke.*[1b]

Since spontaneous decomposition of the acid occurs much more rapidly in the incubator than at room temperature, the *p*H growth range was determined both at 37.5 and 25° in order to detect any discrepancies due to this decomposition; but the growth under these different conditions was practically identical except in rate. Addition of an equivalent of sodium formate had no effect on the *p*H growth range.

Discussion

No satisfactory way to predict whether an organic substance will by itself support bacterial growth is known. Quastel states[2] that to support growth a molecule must fulfil two requirements: it must be activated by the resting organism in the presence of methylene blue, and it must be capable of transformation into pyruvic acid. While some predictions were successfully made on this basis, the only reliable way to tell whether a substance can be converted to pyruvic acid is to isolate this acid from a medium containing the substance. Serious difficulties in interpreting results obtained by the methylene blue technique have been pointed out by Wieland.[15] Hence direct study of growth-supporting abilities of substances is essential.

(15) H. Wieland, *Helv. Chim. Acta*, **15**, 521 (1932).

But studies using the methylene blue technique have given valuable data. Thus the reducing coefficients[16] of the simple fatty acids clearly show an alternation from even to odd,[17] just as the growth of bacteria alternates from even to odd. Alternation of this type is a general phenomenon in nature, for only even-numbered straight chain compounds occur in natural fats,[18] and the physical properties of straight chain substances alternate with the number of carbon atoms.[19] Furthermore, studies of the effect of organic structure on adsorbability[20] show that an alternation in the adsorption of even and odd-numbered acids on Norite occurs and that these substances are presumably adsorbed in the solid state. Comparison with the data given here shows that the odd-numbered monocarboxylic acids which are more strongly adsorbed are also better able to support growth. It appears probable, therefore, that both carbon and bacteria adsorb these substances in a similar way, and that if bacteria utilize the substances thus adsorbed at the cell surface, in accord with Quastel's hypothesis, they may act on materials with characteristics of the solid state.

Since alternation in bacterial growth occurs, the decomposition process bears some resemblance to β-oxidation, in which even-odd alternation also occurs. Knoop[21] states that β-hydroxy and β-keto acids behave like the saturated acid in β-oxidation. Now β-hydroxybutyric acid supports fair growth by *B. aertrycke* and good growth by *B. pyocyaneus;* butyric acid itself supports moderate growth by the former organism and excellent although slower growth by the latter. These facts are evidence that in the initial stages of this decomposition β-oxidation may occur.

The fact that *cis* acids support in general better growth than stereoisomeric *trans* acids shows that the geometric isomerism found in these molecules may be a factor in determining whether bacteria can utilize a substance. The *cis* polybasic acids have both functional groups on the same side of a double bond, whereas the *trans* acids have the

(16) Acid HCOOH, CH_3COOH, CH_3CH_2COOH, $CH_3(CH_2)_2COOH$, $CH_3(CH_2)_3COOH$; Reducing coefficient 700, 0.95, 0.46, 0.64, 0.5.

(17) J. H. Quastel and M. D. Whetham, *Biochem. J.*, **19**, 520 (1925).

(18) P. B. Hawk and O. Bergeim, "Practical Physiological Chemistry," P. Blakiston Sons, Philadelphia, Pa., 1931, p. 175.

(19) J. D. Meyer and E. E. Reid, THIS JOURNAL, **55**, 1574 (1933).

(20) E. R. Linner and R. A. Gortner, *J. Phys. Chem.*, **39**, 35 (1935).

(21) F. Knoop, *Ahrens Sammlung*, **9**, n. f. 12 (1931).

functional groups on opposite sides of the bond. *Cis* compounds in general, moreover, have higher energy content than the corresponding *trans* compounds, shown by lower melting points and higher heats of combustion.[22] By comparing a larger series of these acids, some indication of the importance of the different atoms or groups of atoms in the molecule for the bacterial decomposition can perhaps be secured.

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Summary

1. It is known that in many cases particular species of bacteria can be grown in a medium containing in addition to inorganic salts a single pure organic acid as a source of carbon and energy. Growth in such a medium means that the organism can decompose the acid into fragments which are useful to it, and therefore that this acid may occur in the intermediary metabolism of the organism.

2. The ability of a large number of pure organic acids at a variety of *p*H values to support the growth of different types of bacteria has been recorded.

3. The information thus obtained not only is necessary preliminary information in the study of intermediary metabolism, but it has made possible the following generalizations about the relation of the molecular structures of organic acids and their capacities to support bacterial growth in the absence of any other source of carbon: (a) the *cis* stereoisomer has greater ability to support growth than the *trans* form; (b) among the lower normal fatty acids (1-4 carbon atoms), growth occurs more abundantly in media composed of acids with *even* numbers of carbon atoms than in those with *odd* numbers of carbon atoms; (c) substitution of an hydroxyl for hydrogen in these acids results in a reversal of the relation between the number of carbon atoms and growth for all hydroxy acids, except β-hydroxybutyric acid, which have been examined: growth is poor on *even*-numbered hydroxy acids and abundant on *odd*-numbered hydroxy acids; (d) the amino acids which have been studied show the same relation as the hydroxy acids; (e) growth diminishes as the molecular weight of the acid increases, except for the alternation among the lower fatty acids; (f) substitution of a phenyl group in the molecule greatly diminishes the ability of a substance to support the growth of bacteria; (g) substitution of a methyl group may have either a favorable or an unfavorable effect, according to the position substituted; (h) acids with stable cyclic structures support in general much less growth than acids which do not contain rings; (i) a number of saturated and unsaturated acids are unique in supporting growth in the same range of *p*H for each pair, indicating interconversion of the saturated and the unsaturated acids in bacterial metabolism.

These generalizations are in harmony with the scattered observations which have been found in the literature.

(22) Landolt-Börnstein, "Tabellen," Verlag Julius Springer, Berlin, 1905, p. 422.

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