EFFECT OF KETONE BODIES AND OTHER METABOLITES ON THE SURVIVAL AND MULTIPLICATION OF STAPHYLOCOCCI AND TUBERCLE BACILLI

By RENÉ J. DUBOS, Ph.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

Uncontrolled diabetes, starvation, as well as other conditions resulting in ketosis, are commonly associated with abnormally high susceptibility to various infections, in particular to those caused by staphylococci and tubercle bacilli. The enhancing effect of hyperglycemia on bacterial multiplication, the decreased activity of the phagocytes (claimed to be related to their high glycogen content), some ill defined defect in antibody production, have all been postulated to play some part in the low resistance to infection of the patient with ketosis. These explanations, however, do not appear to be supported by convincing observations or experiments, and they fail, furthermore, to account for many facts of the infectious process.

The present investigation was stimulated by another working hypothesis, namely that infection during ketosis is facilitated by the additive effect of two independent factors; on the one hand, the presence of ketone bodies in abnormally high concentrations; on the other hand, the decreased production of lactic acid at the site of inflammation. Some experiments dealing with the effect of various metabolites on the survival and multiplication of microorganisms *in vitro* will be presented in support of this hypothesis. The design of these experiments was based on the following considerations.

Rationale of the Work

Lactic acid is known to accumulate in inflammatory areas and to be produced by normal leucocytes (1). Experiments in our laboratory have revealed, furthermore, that lactic acid can exert *in vitro* a bacteriostatic and bactericidal effect on tubercle bacilli, particularly in slightly acidic media with reduced oxygen supply. In contrast, it was found that keto acids have a much lower antibacterial activity under the same conditions (2). These observations suggested the possibility that a disturbance in the lactic acid–ketone bodies ratio might create a biochemical environment favorable to infectious agents.

Two reasons made it appear advisable to use acid media to test the validity of this hypothesis:—

(*a*) The intracellular environment of leucocytes has long been known to become very acid immediately following engulfment of a variety of inanimate and living ob-

jects; thus, phagocytic experiments with dyes have revealed that the pH within the leucocytic vacuole may go down to 4.0 or even lower (3). As staphylococci and tubercle bacilli are rapidly phagocytized *in vivo*, it is likely that they are exposed to acid reactions for some time during the intracellular phase of the infectious process.

(*b*) Although no information has been published describing the precise physicochemical environment of inflammatory areas, and in particular their pH, there is much suggestive evidence nevertheless, that the extracellular environment of these areas is also acid, probably as a result of the glycolytic activities of inflammatory cells.

In order to approximate the conditions encountered by microbial agents *in vivo*, experiments were conducted in media consisting of balanced ionic solutions (of the type used in tissue culture), to which serum albumin was added in final concentration of 0.5 per cent and which had been adjusted to acid reactions ranging from neutrality to pH 4.0.

The results obtained in the present study demonstrate that staphylococci and tubercle bacilli die very rapidly at acid reactions in media containing lactic acid in a concentration of 0.01 M (or smaller); acetic, propionic, and butyric acids exert a similar, but less pronounced bactericidal effect. Under the same conditions, the microorganisms survive longer in the presence of polybasic acids (succinic, fumaric, and citric) and of glutamic acid, and more particularly if ketone bodies such as dihydroxyacetone, pyruvic acid, β-hydroxybutyric acid, α-ketoglutaric acid, and oxalacetic acid are added to the suspension fluid. Indeed, ketone bodies can neutralize in part the antimicrobial effect of lactic acid and furthermore, their addition to culture media permits microbial growth at acid reactions (approximately pH 5.3).

Extensive experiments *in vitro* with eight strains of staphylococci and six strains of tubercle bacilli (human and bovine), confirmed by preliminary tests with other microbial species, have thus brought out the fact that lactic acid antagonizes, and ketone bodies favor, the survival and multiplication of certain pathogenic microorganisms in certain acid environments. These opposite effects of the two groups of metabolites suggest that the metabolic disturbances of ketosis may bring about increased susceptibility to infection through two distinct, but perhaps related mechanisms: on the one hand, by depressing the antimicrobial effect of lactic acid at the site of inflammation; on the other, by providing to the infectious agents ketone bodies which act as growth-enhancing factors.

EXPERIMENTAL

Cultures.—The six strains of mammalian tubercle bacilli used in this study have been described in preceding publications under the labels MV, BCG-P, BCG-T, H37Rv, R1Rv, and H37Ra (4). Stock cultures were incubated for 8 to 12 days at 37°C. in the tween-albumin medium commonly in use in our laboratory (5).

Of the eight cultures of staphylococcus that were tested, four were pigmented (orange or lemon yellow) and coagulase-positive, the other four produced white colonies and were coagulase-negative. All were maintained in meat infusion–peptone broth.

In general, it appeared that the avirulent cultures (of tubercle bacilli, and particularly of staphylococci) were more susceptible to the lethal effects of acid reactions than were the virulent cultures. Nevertheless, the general pattern of behavior toward the various metabolites tested was remarkably similar for all cultures. For the sake of brevity, actual results will be presented for only two cultures, namely, "BCG-P" (an attenuated strain of *Mycobacterium tuberculosis bovis*) and "Smith," a pigmented coagulase-positive strain of *Micrococcus aureus*, originally isolated from a patient with osteomyelitis.

Materials.—Most of the substances tested were obtained from commercial sources: Acetic acid (Mallinckrodt Chemical Co., St. Louis, Missouri); butyric and propionic acids (Amend Drug and Chemical Co., Inc., New York); citric and lactic acids (Merck and Co., Inc., Rahway, New Jersey); fumaric acid (Chas. Pfizer and Co., Brooklyn, New York); pyruvic, β-hydroxybutyric, and α-ketoglutaric acids (The Matheson Co., Inc., East Rutherford, New Jersey); glutamic acid (Eastman Kodak Co., Rochester, New York); oxalacetic acid (Krishell Laboratory, Inc., Portland, Oregon).

Confirmatory experiments were carried out also with purified samples of the three following substances: L+ lactic acid (obtained from Dr. Thompson of Ohio State University through the courtesy of Dr. L. H. Geronimus); pyruvic acid and dihydroxyacetone recrystallized and kindly supplied by Dr. R. D. Hotchkiss).

Stock solutions of the different substances were made in distilled water, brought to the desired pH with NaOH, made up to 0.25 M concentration, and sterilized. The solutions of acetate, propionate, butyrate, succinate, fumarate, citrate, glutamate, and lactate were sterilized by autoclaving at 15 pounds' pressure for 10 minutes. On account of their low stability, the solutions of pyruvate, β-hydroxybutyrate, α-ketoglutarate, oxalacetate, and dihydroxyacetone, were prepared immediately before use and were sterilized not by heating, but by filtration through porcelain (Selas) candles. In a few experiments even filtration was omitted in order to minimize any chance of alteration of these substances. The samples were rapidly weighed, dissolved in sterile water, and the reaction of the solution was adjusted by adding to it the required amount of NaOH in the presence of a pH indicator.

Except as indicated in the text, the experiments were conducted in a balanced ionic medium of the following composition:—

NaCl	13.6 gm.
KCl	0.8 "
$MgSO_4 \cdot 7H_2O$	0.2 "
$NaH_2PO_4 \cdot H_2O$	0.29 "
$CaCl_2$	1 drop, 10 per cent
Water	100.0 ml.

The medium was prepared by adding 5 ml. of this stock solution to 95 ml. of distilled water, and adjusting the pH to the desired levels.

Serum albumin (bovine plasma fraction V obtained from the Armour laboratory, Chicago), was added in the form of a 5 per cent solution in saline adjusted to the proper pH and sterilized by filtration through porcelain candles (Selas).

Performance of Tests.—All components of the test medium were adjusted at the proper pH and mixed aseptically. The bacterial suspensions were diluted in 5 per cent albumin (at the proper pH) and added in one-tenth volume to the mixture (giving a final concentration of 0.5 per cent albumin). The final pH was determined by testing an aliquot portion of the mixture with a Beckman glass electrode pH meter. In many experiments, the pH of the mixture was determined again at the end of the incubation period. This was particularly necessary in the case of mixtures containing ketone bodies, since these substances are unstable and undergo alteration rapidly in aqueous medium at 37°C. By using low concentrations of the

ketone bodies in buffered media, it was possible to arrange that the reaction did not change by more than 0.1 pH unit in the course of short term experiments. The only exception was that of oxalacetic acid. This substance decomposed so rapidly under the conditions of the test that only qualitative observations could be made; for this reason, results obtained with oxalacetic acid are not included in the tables.

The time of survival and extent of multiplication of the microorganisms were determined by plating dilutions of the test bacterial suspensions after various incubation periods. Meat infusion–peptone agar was used for staphylococci, and asparagine-albumin agar (5) for tubercle bacilli. The dilutions of staphylococci were made in meat infusion–peptone broth and one loopful of each dilution was streaked on the agar surface. The dilutions of tubercle bacilli were made in albumin solution (without tween) and 1 drop (of approximately 0.025 ml. volume) was deposited from a Pasteur capillary pipette on the agar surface. All dilutions were selected to give well isolated colonies that could be counted (after 24 to 48 hours' incubation for staphylococci, after 2 to 3 weeks' incubation for tubercle bacilli). The numbers of viable units entered in the tables refer to the number of colonies recovered per milliliter of bacterial suspension in the test medium, determined by calculation from the number of colonies actually yielded on agar plates by each dilution.

RESULTS

Viability of Staphylococci and Tubercle Bacilli at pH 4.0 and pH 5.5

To a number of tubes there were added aseptically 2.0 ml. of the balanced mineral solution and 0.2 ml. of 0.25 M (for tubercle bacilli) or 0.1 M (for staphylococci) solutions of the various substances listed in Tables I and II. Two media were used in each case, one at pH 4.0, the other at pH 5.5. The tubes were inoculated with 0.2 ml. of culture of BCG-P (10 days old) or of staphylococcus Smith (2 days old) diluted 1,000-fold in 5 per cent albumin (at pH 4.0 and at pH 5.5). In order to minimize evaporation, the cotton plugs of the tubes containing tubercle bacilli were sealed with paraffin. All bacterial suspensions were placed at 37°C. and the numbers of living organisms determined by plating on agar after 1, 2, 3, 5, 24, and 48 hours in the case of staphylococci, and 1, 2, 3, 5, 7, 10, and 14 days in the case of tubercle bacilli. The numbers of viable units (calculated per milliliter of bacterial suspension) are indicated in Tables I and II.

As was to be expected, the tubercle bacilli survived much longer than the staphylococci and the microorganisms of both groups died more rapidly at pH 4.0 than at pH 5.5. Except for a few minor exceptions (particularly with reference to their behavior toward glutamic acid) both groups of microorganisms exhibited the same pattern of susceptibility to the various substances tested. Survival at both reactions was much shorter in the solutions containing lactic acid than in the solutions with no organic acid added. Acetic, butyric, and propionic also exerted a bactericidal effect, but less rapid than that of lactic acid. Succinic, fumaric, and citric acid proved less toxic than lactic and the aliphatic acids. Glutamic acid was remarkably innocuous for tubercle bacilli, but somewhat less for staphylococci. Survival was longest in tubes containing dihydroxyacetone, pyruvic, β-hydroxybutyric, α-ketoglutaric, and oxalacetic acids. Unfortunately, the pH of the solutions of oxalacetic acid increased in the course of incubation and for this reason it was not considered justifiable to include in the tables the results obtained with this substance.

TABLE I

Effect of Various Metabolites on Viability of Staphylococci at Acid Reactions

Substance added to suspension of staphylococci (Final concentration 0.01 M)		No. of viable staphylococci* after exposure at							
		pH 4.0 for (hrs.)				pH 5.5 for (hrs.)			
		1	2	3	5	2	5	24	48
Control		3×10^5	1×10^5	6×10^4	1×10^4	3×10^5			2×10^5
Acetic	acid	2×10^5	3×10^4	0	0	1×10^5	1×10^4	0	0
Propionic	"	2×10^5	3×10^4	1×10^4	0	2×10^5	1×10^5	1×10^4	
Butyric	"	3×10^5	8×10^4	3×10^4	0	3×10^5	1×10^5	2×10^4	
Succinic	"	3×10^5	2×10^5	1×10^5	0	3×10^5	2×10^5	5×10^4	
Fumaric	"	3×10^5	2×10^5	1×10^5	0	3×10^5	3×10^5	3×10^4	
Citric	"	3×10^5	2×10^5	6×10^4	0	3×10^5	2×10^5	4×10^4	
Lactic	"	1×10^5	2×10^4	0	0	5×10^4	0	0	
Pyruvic	"	3×10^5	3×10^5	1×10^5	6×10^4	3×10^5	4×10^5	1×10^6	5×10^6
β-Hydroxybutyric	"	3×10^5	2×10^5	9×10^4	1×10^4	3×10^5	2×10^5	2×10^5	1×10^6
α-Ketoglutaric	"	3×10^5	3×10^5	2×10^5	1×10^5	3×10^5	3×10^5	4×10^5	7×10^5
Glutamic	"					3×10^5	2×10^5	2×10^4	9×10^3
Dihydroxyacetone		3×10^5	3×10^5	3×10^5	2×10^5	3×10^5	4×10^5	4×10^5	6×10^6

* Determined by duplicate plating on meat infusion–peptone agar.

TABLE II

Effect of Various Metabolites on Viability of Tubercle Bacilli at Acid Reactions

Substance added to suspension of bacilli (Final concentration 0.025 M)		No. of viable bacilli* after exposure at									
		pH 4.0 for (days)					pH 5.5 for (days)				
		1	2	3	6	8	3	5	7	10	14
Control		10^5	10^5	10^5	10^3	0	10^5	10^5	10^5	10^5	10^4
Acetic	acid	10^4	10^3	0	0	0	10^2	0	0	0	0
Propionic	"	10^4	10^3	0	0	0	10^5	10^5	10^4	10^3	0
Butyric	"	10^4	10^3	0	0	0	10^5	10^5	10^4	10^4	0
Succinic	"	10^5	10^5	10^4	0	0	10^5	10^5	10^5	10^4	10^2
Fumaric	"	10^5	10^5	10^4	0	0	10^5	10^5	10^5	10^6	10^7
Citric	"	10^5	10^5	10^5	0	0	10^5	10^5	10^5	10^5	10^4
Lactic	"	10^4	0	0	0	0	0	0	0	0	0
Pyruvic	"	10^5	10^5	10^5	10^4	10^2	10^5	10^5	10^5	10^4	10^3
β-Hydroxybutyric	"	10^5	10^5	10^3	0	0	10^5	10^5	10^5	10^5	10^3
α-Ketoglutaric	"	10^5	10^5	10^5	10^2	0	10^5	10^5	10^6	10^3	10^3
Glutamic	"	10^5	10^5	10^5	0	0	10^5	10^5	10^6	10^3	10^3
Dihydroxyacetone		10^5	10^5	10^5	0	0	10^5	10^5	10^5		
Glycine		10^5	10^5	10^5	10^2	0	10^5	10^5	10^5	10^4	10^3
Alanine		10^5	10^5	10^4	0	0	10^5	10^5	10^4	10^3	10^2

* Determined by duplicate plating on albumin–asparagine agar. Calculated to the nearest exponential value of 10.

TABLE III

Effect of Various Metabolites on Toxicity of Acetic and Lactic Acids for Staphylococci at Acid Reactions

Substances added to suspension of staphylococci (Final concentration 0.02 M)	No. of viable staphylococci* after exposure at								
	pH 4 for (*hrs.*)				pH 5.5 for (*hrs.*)				
	2	3	4	5	3	5	8	24	48
Acetic acid	1 × 10⁵	4 × 10⁴	0	0	3 × 10⁴	0	0	0	0
" " + pyruvic acid	3 × 10⁵	1 × 10⁵	7 × 10⁴	0	2 × 10⁵	3 × 10⁴	0	0	0
" " + α-ketoglutaric "					3 × 10⁵	2 × 10⁵	9 × 10⁴	4 × 10⁴	0
" " + β-hydroxybutyric "	8 × 10⁴	3 × 10⁴	0	0	2 × 10⁵	8 × 10⁴	2 × 10⁴	0	0
" " + dihydroxyacetone	2 × 10⁵	3 × 10⁴	1 × 10⁴		7 × 10⁴	1 × 10⁴	0	0	0
Lactic acid	2 × 10⁴	0	0	0	4 × 10⁴	0	0	0	0
" " + pyruvic acid	9 × 10⁴	2 × 10⁴	0	0	2 × 10⁵	3 × 10⁴	0	0	0
" " + α-ketoglutaric "					3 × 10⁵	1 × 10⁵	7 × 10⁴	2 × 10⁴	1 × 10⁴
" " + β-hydroxybutyric "	2 × 10⁴	5 × 10³	0	0	3 × 10⁵	2 × 10⁵	5 × 10⁴	0	0
" " + dihydroxyacetone	9 × 10⁴	3 × 10⁴	0	0	1 × 10⁵	4 × 10⁴	0	0	0

* Determined by duplicate plating on meat infusion–peptone agar.

TABLE IV

Effect of Various Metabolites on Toxicity of Acetic and Lactic Acids for Tubercle Bacilli at Acid Reactions

Substances added to suspension of bacilli (Final concentration 0.025 M)	No. of viable bacilli* after exposure at							
	pH 4 for (*days*)				pH 5.5 for (*days*)			
	1	2	3	6	3	5	7	10
Acetic acid	9 × 10⁴	7 × 10³	0	0	8 × 10³	0	0	0
" " + pyruvic acid	2 × 10⁵	5 × 10⁴	0	0	9 × 10³	3 × 10³	0	0
" " + α-ketoglutaric "	3 × 10⁵	1 × 10⁵	4 × 10⁴	2 × 10³	3 × 10⁵	2 × 10⁵	7 × 10⁴	1 × 10⁴
" " + β-hydroxybutyric "	2 × 10⁵	3 × 10⁴	0	0	1 × 10⁵	1 × 10⁴	0	0
" " + glutamic "					2 × 10⁵	2 × 10⁵	4 × 10⁵	7 × 10⁵
Lactic acid	4 × 10⁴	0	0	0	2 × 10⁴	0	0	0
" " + pyruvic acid	1 × 10⁵	6 × 10³	0	0	0	0	0	0
" " + α-ketoglutaric "	3 × 10⁵	4 × 10⁴	0	0	2 × 10⁵	1 × 10⁵	0	0
" " + β-hydroxybutyric "	8 × 10⁴	4 × 10³	0	0	5 × 10³	2 × 10³	0	0
" " + glutamic "					1 × 10⁵	2 × 10⁵	4 × 10³	9 × 10⁵
" " + dihydroxyacetone					2 × 10⁵	4 × 10⁵	1 × 10⁶	3 × 10⁶

* Determined by quadruplicate plating on asparagine–albumin agar.

Partial Neutralization of the Antibacterial Effect of Lactic and Acetic Acids by Various Metabolites

Tubes containing 2 ml. of the balanced ionic solution with 0.025 M of either acetic or lactic acid, at pH 4.0 and pH 5.5, received in addition 0.2 ml. of physiological saline, or of 0.25 M solutions of dihydroxyacetone, or pyruvic, β-hydroxybutyric, α-ketoglutaric, or oxal-acetic acid. The tubes were then inoculated with 0.2 ml. of suspensions of staphylococci, or of tubercle bacilli, that had been diluted 1000-fold in 5 per cent albumin at pH 4.0 or pH 5.5.

The viability of the organisms was tested after various incubation periods, as described in the preceding experiment. The numbers of viable units (calculated per milliliter of suspension) are given in Tables III and IV.

The results presented in Tables III and IV reveal that the survival time of staphylococci and tubercle bacilli in the presence of acetate and lactate at acid reaction was slightly increased by addition of ketone bodies to the test systems. Of the substances listed in the tables, α-ketoglutaric acid proved to be most effective in its protective effect. Even more satisfactory results were obtained with oxalacetic acid. Unfortunately, as already observed in the case of the preceding experiment, the pH of the media containing this substance increased during incubation, thus preventing adequate quantitative comparisons.

TABLE V

Effect of Age of Culture on Susceptibility of Staphylococci to Lactic Acid

Age of culture	Final concentration of lactic acid	No. of viable staphylococci* after exposure at pH 4.0 for (*hrs.*)		
		4	10	16
6 hrs.	0	2×10^4	3×10^3	0
"	0.01 M	7×10^2	8×10^1	0
"	0.025 M	5×10^2	8×10^1	0
8 days	0	1×10^5	8×10^4	6×10^3
"	0.01 M	7×10^4	5×10^3	2×10^2
"	0.025 M	5×10^4	6×10^3	9×10^1

* Determined by duplicate plating on meat infusion–peptone agar.

Effect of Age of the Culture on the Survival of Staphylococci at Acid Reactions

In the course of the many tests that were performed in order to determine the effect of various metabolites on the viability of staphylococci at acid reactions, it was observed that—with any given strain—the rate of death in the tubes having received the test substances, as well as in control tubes, differed markedly from one experiment to the other. As every effort had been made to carry out the tests under identical environmental conditions, it appeared likely that the origin of the differences in results resided in the staphylococci themselves. Among the many factors that might influence the susceptibility of staphylococci to the bactericidal effect of acid media, only one has been studied so far, namely the age of the culture.

Two cultures of the "Smith" strain of staphylococcus were compared, one 6 hours old still in the logarithmic period of growth, the other 8 days old. The latter was stored at night in the refrigerator throughout the week, but kept at room temperature for several hours every day during that period. The viability of the two cultures in the presence and absence of lactic acid was determined by techniques similar to those used in the two preceding experiments.

The results presented in Table V make clear that the staphylococci of the older culture (8 days) survived much longer than did the organisms in the logarithmic phase of growth whether or not lactic acid was present in the suspension fluid at pH 4.0. In other tests not reported here, it was found that the older staphylococci did survive for several weeks at pH 5.5, whereas the young cells died within a very few days under the same conditions. Unfortunately, technical problems have made it difficult so far to determine the effect of age of the culture on the resistance of tubercle bacilli to various metabolites in acid media.

Effect of Various Metabolites on the Multiplication of Staphylococci and Tubercle Bacilli at Acid Reactions

The results presented in Tables I and II suggest that, at pH 5.5, there took place some multiplication of the staphylococci and tubercle bacilli resuspended in solutions containing ketone bodies or glutamic acid. Experiments were, therefore, instituted to ascertain this fact in a more convincing manner.

A basal medium of the following composition was prepared:

0.5 M Na_2HPO_4	50.0 ml.
0.5 M KH_2PO_4	5.0 "
0.05 M $MgSO_4$	6.5 "
0.05 M $CaCl_2$	6.5 "
3 per cent yeast extract	6.5 "
H_2O to	650.0 "

Aliquots of this medium were adjusted to reactions ranging from pH 7.0 to pH 4.5. After sterilization by autoclaving, serum albumin (at pH 5.5) was added aseptically in a final concentration of 0.5 per cent. The albumin media were then distributed in 3 ml. amounts in a number of tubes to each of which was added 0.3 ml. of oxalacetic acid or of solutions of the various metabolites listed in Tables VI and VII. These had been adjusted to reactions ranging from pH 4.5 to 7.0 before addition to the media of the corresponding pH.

The tubes were inoculated with 0.3 ml. of staphylococcus cultures (2 days old) diluted 100-fold in 2 per cent glucose solution in water. In a similar experiment the tubes of a duplicate set were inoculated with 0.3 ml. of culture of tubercle bacilli diluted 10-fold in glucose solution. The cultures were incubated at 37°C. and the extent of growth was estimated after 12 hours' incubation for the staphylococci, and 4 days' for the tubercle bacilli.

The pH of each of the media was determined (by use of the glass electrode) on an aliquot before incubation, and again at the end of the experiment. Only data referring to media, the reaction of which did not change by more than 0.2 pH unit, are included in Tables VI and VII. Because of the large numbers of variables involved, it was not practically possible to perform all the tests simultaneously. In consequence, Tables VI and VII present composites of the results of several experiments.

Several conclusions appear obvious from examination of Tables VI and VII. It can be seen that the minimum and optimum pH for growth depended not only upon the species of organism used, but even more upon the composition of the medium. With staphylococci for example, growth took place at pH 5.3 in the pyruvic acid medium, but only at reactions near neutrality in the lactic

and acetic acid media. Similarly, growth of tubercle bacilli was observed at a pH as low as 5.2, in media containing α-ketoglutaric acid, dihydroxyacetone,

TABLE VI

Effect of Various Metabolites on the Multiplication of Staphylococci at Acid Reactions

Substance added to the medium (Final concentration 0.025 M)		Growth* after 18 hrs.' incubation (37°C.) at indicated pH			
		5.3	5.5	5.8	6.9
0		−	++	++++	+++
Acetic	acid	−	−	−	+++
Propionic	"	−	−	−	
Butyric	"	−	−	−	
Lactic	"	−	+	++	++++
Fumaric	"	−	−	+++	++++
Pyruvic	"	++	++++	++++	+++
α-Ketoglutaric	"	−	+	+++	++++
β-Hydroxybutyric	"	+	++	+++	++
Glutamic	"	−	++	++	++++
Dihydroxyacetone			++++		

* Recorded according to an arbitrary scale (visual observation) from − (no growth) to ++++ (maximum growth).

TABLE VII

Effect of Various Metabolites on the Multiplication of Tubercle Bacilli at Acid Reactions

Substance added to the medium (Final concentration 0.025 M)		Growth* after 4 days' incubation (37°C.) at indicated pH					
		4.5	5.2	5.5	5.8	6.4	6.9
0		−	−	++	++	+++	++
Acetic	acid	−	−	−	−		+++
Propionic	"	−	−	−	−		+++
Butyric	"	−	−	−	−		+++
Lactic	"	−	−	−	−	+++	++++
Fumaric	"	−	−	++++	++++	++++	++++
Pyruvic	"	−	−	−	++	++++	+++
β-Hydroxybutyric	"	−	−	−	+++	++++	++
α-Ketoglutaric	"	−	++	++++	++++		++
Glutamic	"	−	+++	++++	++++	++++	++++
Dihydroxyacetone		−	++	+++	+++	+	−

* Recorded according to an arbitrary scale (visual observation) from − (no growth) to ++++ (maximum growth).

and glutamic acid but only at reactions nearer to neutrality in the presence of acetic, propionic, butyric, and lactic acids. Furthermore, lactic acid which was inhibitory to the growth of both types of microorganisms, at acid reactions, did enhance their growth at neutrality. The opposite situation was observed

with dihydroxyacetone, which enhanced the growth of tubercle bacilli at acid reactions, and inhibited it at reactions higher than pH 6.5. (As dihydroxyacetone is unstable under the conditions of the test, it is possible that this inhibitory effect was due not to dihydroxyacetone itself, but rather to its decomposition products at or near neutrality.)

Thus, growth experiments reveal that lactic acid (and also some short aliphatic acids) inhibit, and ketone bodies stimulate, the multiplication of staphylococci and tubercle bacilli at the acid reactions which can be assumed to prevail in inflammatory areas. These findings in culture media confirm and extend those of the viability tests performed in mineral solutions. Needless to say, there are many complex factors which are bound to alter the play of these biochemical forces *in vivo*. The results of the present study make it appear likely nevertheless that ketosis, as well as other metabolic disturbances, can exert a profound influence on the course of infection, by affecting the survival and multiplication of microorganisms through changes in the biochemical environment—local or general, intracellular or extracellular.

SUMMARY

A study has been made of the fate of staphylococci and tubercle bacilli resuspended in aqueous media at slightly acid reactions. The tests were carried out at several acid reactions in balanced ionic media containing 0.5 per cent serum albumin. These experimental conditions were selected in order to approximate those which are probably encountered by pathogenic agents in inflammatory areas and in the intracellular environment of the leucocytes after phagocytosis.

The viability of the microorganisms at a given pH was markedly influenced by the composition of the medium, being decreased by addition to the latter of lactic, acetic, propionic, and butyric acids, and increased by the addition of certain ketone bodies such as dihydroxyacetone and pyruvic, β-hydroxybutyric, α-ketoglutaric, and oxalacetic acids.

The presence of ketone bodies in the medium afforded to the microorganisms some protection against the bactericidal effect of lactic and acetic acids at acid reactions.

The minimum and the optimum pH for growth were found to be dependent on the composition of the medium. Both were higher in the presence of lactic, acetic, propionic, and butyric acids than in the media without organic acids added. In contrast, the addition of ketone bodies to the medium allowed microbial multiplication even in acid media (approximately at pH 5.3 or even lower).

The fact that lactic acid antagonizes, whereas ketone bodies favor, the survival and multiplication of staphylococci and tubercle bacilli at acid reactions, is discussed in relation to the high susceptibility to infection which is often associated with ketosis of various etiology.

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