Lactic Acid Utilization by the Cutaneous Micrococcaceae

RODNEY F. SMITH

Department of Microbiology, Temple University Dental School, Philadelphia, Pennsylvania 19140

Received for publication 25 September 1970

Human cutaneous staphylococci and micrococci utilized lactic acid as an energy source on a minimal medium. Propionic acid was not utilized, but L(−)-lactic acid and pyruvic acid could replace LD-lactic acid as a substrate. Selected strains of cocci were inhibited more by the L(+) and D(−) forms of lactic acid than the balanced LD form, particularly at pH 5.6. With proper dilution of substrate, lactic acid was utilized by selected strains in the presence of 10 μg of oleic and palmitic acids per ml.

Numerous dietary and other salivary-derived carbohydrates are available to the facultative oral bacteria as energy sources, whereas simple sugars are absent from the secretions of the epidermis (3). This study was initiated to determine whether lactic acid has a role in the nutrition of the predominant gram-positive facultative cutaneous bacteria.

Cutaneous samples were obtained by using the swabbing techniques previously reported (5). Gram-positive catalase-producing cocci were separated into groups of staphylococci and micrococci by the methods of Baird-Parker (1). Nutrient agar slants (Difco) were used to maintain the isolates. Isolation and enumeration of skin bacteria were done with Trypticase Soy Agar without glucose (BBL) and a minimal medium with the following composition per liter: NH₄Cl, 1.0 g; K₂HPO₄, 7.0 g; KH₂PO₄, 3.0 g; MgSO₄·7H₂O, 0.2 g; MnCl₂, 10 mg; FeCl₃·6 H₂O, 1 mg; NaCl, 1.0 g; CaCl₂, 1 mg; and yeast extract (Difco), 0.5 g. The final pH was 7.0. The minimal medium was also prepared with a pH of 5.6 by using 0.5 g of K₂HPO₄ and 9.5 g of KH₂PO₄ per liter. Solid minimal medium was prepared by adding 1.5% Noble agar (Difco). Selection of lactic acid-utilizing bacteria was made by plating skin swabblings on the Trypticase and minimal agars with and without the addition of 0.25% optically balanced LD-sodium lactate (Sigma Chemical Co., St. Louis, Mo.). Filter-sterilized pure L(+) and D(−)-lactic acids (Mann Research Laboratories, New York, N.Y.) were added to media after sterilization. Sodium pyruvate, sodium propionate, and sodium acetate were sterilized with media. All media were autoclaved at 121°C for 15 min. Sodium oleate and palmitic acid (Sigma) were each dissolved in 95% ethanol and added to media after sterilization. Lactic acid was measured colorimetrically (2) by measuring the quantity of substrate depleted from broth which was compared to controls to express the amounts of substrate utilized. Acetate-/lactic acid production was determined with 5% alcoholic alpha naphthol and KOH-creatine reagents.

Cultures were incubated in 125 by 16 mm screw-capped tubes under stationary conditions or shaken on a metaboIyte water bath (New Brunswick Scientific Co.) at 200 rev/min. All experiments were conducted aerobically at 34°C. Optical density of broth cultures was measured with the Bausch & Lomb Spectronic-20 colorimeter. Inoculum was washed off nutrient slants in phosphate-buffered saline and adjusted to an optical density of 0.5 at 620 nm. A 0.05-ml quantity of this cell suspension was used to inoculate 4 ml of broth.

When cutaneous samples were plated on Trypticase Soy Agar with and without 0.25% LD-lactate, little or no differences in total aerobic counts were observed on these two media although the size of all the colonies on the lactate-containing medium was generally larger. By using minimal medium, the numbers of bacteria from each of 18 samples were consistently greater and colony size was larger on minimal lactate agar when compared to basal minimal medium (Table 1 and Fig. 1). Bacterial populations from some sites did not grow on minimal medium unless lactate was added. Four additional samples from the scalp, forehead, groin, and toeweb were plated on minimal media containing 0.2% LD-lactate, L(−)-lactic, pyruvic, and propionic acids. This experiment indicated that pyruvic and L(−)-lactic acid could replace LD-lactate as substrates for the skin flora, but propionic acid was not utilized. Since the media did not contain significant amounts of lipid, the cutaneous lipophilic diphtheroids were
either absent or grew as pin-point colonies. When observed, they were counted on Trypticase Soy Agar as part of the total count which was the reason that total counts from some samples were much higher than those on minimal lactate medium. Neither the number nor the colony size of the diphtheroids increased on the lactate medium. The lactate-utilizing bacteria were essentially all coagulase-negative staphylococci or micrococci. Forty of these strains were isolated from minimal lactate medium and transferred to the minimal lactate broth to confirm lactate utilization. Certain strains were selected for further study. Six staphylococcal strains were tested for utilization of L- and propionic acids at pH 5.6 (Table 2). Lactic and pyruvic acids were utilized and acetylmethylcarbinol was produced by the strains in these broths. Acetylmethylcarbinol was not produced in basal, acetic, or propionic broths. Utilization of more than 50% of the LD-lactic acid indicated that both isomeric forms of lactate were used to some extent. When 10 μg of oleic or palmitic acid per ml was added to minimal medium at pH 5.6 containing 0.1% LD-lactic acid, the substrate was also utilized from 50% to 98% by four staphylococci and five micrococal strains. At pH 5.6, 0.1% L(+) and D(-)-lactic acids were inhibitory to the strains in minimal medium. At 0.025%, nine strains of staphylococci and micrococci were stimulated by L(+)-lactic acid, and the substrate was utilized (Table 3). Increasing the substrate
### Table 1. Enumeration of total and lactic acid-utilizing aerobic cutaneous bacteria

| Site    | Sample | Total       | Basal count per swabbing minimal medium | With 0.25% L-lactate |
|---------|--------|-------------|-----------------------------------------|---------------------|
| Scalp.. | 1      | 30,000      | 15,000                                  | 31,000              |
| Forehead| 2      | 135,000     | <500                                    | 135,000             |
|         | 3      | 19,000      | 9,000                                   | 9,500               |
| Nasal.. | 4      | 400,000     | 75,000                                  | 300,000             |
|         | 5      | 1,400,000   | <500                                    | 595,000             |
|         | 6      | 1,000,000   | 330,000                                 | 1,080,000           |
|         | 7      | 3,550,000   | 70,000                                  | 3,800,000           |
|         | 8      | 31,150,000  | 1,400,000                               | 2,150,000           |
| Groin.. | 9      | 12,500,000  | 160,000                                 | 205,000             |
|         | 10     | 2,200,000   | 270,000                                 | 620,000             |
|         | 11     | 3,200,000   | 250,000                                 | 325,000             |
| Toeweb. | 12     | 42,000,000  | 18,000,000                              | 22,000,000          |
|         | 13     | 3,000,000   | <500                                    | 415,000             |
|         | 14     | 210,000     | <500                                    | 330,000             |
|         | 15     | 2,700,000   | 1,600,000                               | 2,700,000           |
|         | 16     | 2,200,000   | 3,500                                   | 50,000              |
|         | 17     | 862,000     | 20,000                                  | 800,000             |
|         | 18     | 1,600,000   | 70,000                                  | 320,000             |

* All media (pH 7.0) were incubated for 4 days. Total counts were made on Trypticase Soy Agar without glucose. Samples were taken from seven normal adult subjects over a period of 4 months.

### Table 2. Comparative utilization of four organic acids by selected strains of cutaneous staphylococci

| 0.1% Substrate | Optical density at 420 nm for strain no. |
|----------------|-----------------------------------------|
|                | 35  | 52  | 30  | 1   | 27  | 42  |
| Control        | 0.68| 0.55| 0.70| 0.60| 0.56| 0.54|
| L-lactic acid  | 1.15| 1.22| 1.10| 0.92| 1.00| 0.95|
| Pyruvic acid   | 0.80| 0.82| 0.81| 0.82| 0.80| 0.85|
| Propionic acid | 0.52| 0.50| 0.57| 0.55| 0.52| 0.43|
| Acetic acid    | 0.52| 0.52| 0.64| 0.54| 0.51| 0.57|
| Lactic acid utilized (%) | 75 | 76 | 75 | 63 | 87 | 99 |

* Cultures were grown in minimal broth (pH 5.6) and shaken in a water bath for 24 hr.

### Table 3. Utilization of L+ lactic acid by cutaneous Micrococcaceae

| Strain | Optical density (420 nm) with Basal medium | Amt of substrate utilized (%) |
|--------|-------------------------------------------|------------------------------|
|        | Basal medium                              | Basal medium with 0.025% lactate |
| S-35   | 0.48                                       | 0.62                         | 72 |
| S-52   | 0.44                                       | 0.70                         | 74 |
| S-1    | 0.51                                       | 0.62                         | 72 |
| S-27   | 0.48                                       | 0.62                         | 60 |
| S-42   | 0.46                                       | 0.58                         | 76 |
| S-8    | 0.16                                       | 0.58                         | 75 |
| S-41   | 0.38                                       | 0.51                         | 70 |
| S-30   | 0.48                                       | 0.62                         | 68 |
| M-47   | 0.09                                       | 0.28                         | 73 |

* S, staphylococci; M, microccci. Cultures were incubated for 21 hr in a shaking water bath.

The results of this study have some dermatological implications. The cutaneous cocci utilized lactate at pH 5.6, the commonly recorded pH of many skin sites (4), in the presence of oleic and palmitic acids which are abundant in epidermal lipids (6). Utilization of L(+)-lactic acid is also of interest because it is the enantiomer of lactate formed from muscular metabolism (7). More detailed metabolic studies are in progress.

### Literature Cited

1. Baird-Parker, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. 38:409-427.
2. Barker, S. B., and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535-554.
3. Miller, P. D. 1969. The carbohydrate metabolism of skin. Brit. J. Dermatol. 81:4-17.
4. Noble, W. C. 1968. Observations on the surface flora of the skin and on the skin pH. Brit. J. Dermatol. 80:279-281.
5. Smith, R. F. 1970. Comparative enumeration of lipophilic and nonlipophilic cutaneous diphtheroids and cocci. Appl. Microbiol. 19:254-258.
6. Smith, R. F. 1970. Fatty acid requirements of human cutaneous lipophilic corynebacteria. J. Gen. Microbiol. 66:259-263.
7. Sokatch, J. R. 1969. Bacterial physiology and metabolism. Academic Press Inc., New York.