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Influence of the pH-Value on the Growth of Staphylococcus epidermidis, Staphylococcus aureus and Propionibacterium acnes in Continuous Culture

Einfluß des pH-Wertes auf das Wachstum von Staphylococcus epidermidis, Staphylococcus aureus und Propionibacterium acnes in kontinuierlicher Kultur

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With 7 Figures

Abstract

A cutaneous isolate of *Staphylococcus epidermidis, Staphylococcus aureus* and *Propionibacterium acnes* was grown in continuous culture at varying pH-values ranging from 5.0 to 8.5. In terms of the specific growth rate as well as the bacterial density during the plateau phase there were remarkable differences. In particular, *Propionibacterium acnes* grew much better in the pH 6.0 to 7.0 range than in a more acidic or alkaline milieu. *Staphylococcus epidermidis* resembled *Staphylococcus aureus* showing no major difference at pH 5.5 and 7.0. These findings substantiate the hypothesis that minor changes of the pH in the pH 5.5 to pH 6.0 range as to be induced by chemically neutral or alkaline skin cleansers on the human skin surface can increase the number of propionibacteria but not staphylococci remarkably due to the relative alkalinity by itself.

Zusammenfassung

Je ein Hautisolat von *Staphylococcus epidermidis, Staphylococcus aureus* und *Propionibacterium acnes* wurde in kontinuierlicher Kultur bei unterschiedlichen pH-Werten gezüchtet, die von 5,0 bis 8,5 reichten. Gemessen an der spezifischen Wachstumsrate sowie der Bakteriendichte während der Plateau-Phase ergaben sich beachtliche Unterschiede. Insbesondere wuchs *Propionibacterium acnes* wesentlich besser im Bereich von pH 6,0 bis 7,0 als in einem saureren oder alkalischeren Milieu. *Staphylococcus epidermidis* und *Staphylococcus aureus* verhielten sich im wesentlichen ähnlich bei pH 5,5 und 7,0. Diese Ergebnisse

untermauern die Hypothese, daß kleinere pH-Veränderungen im Bereich von pH 5,5 bis 6,0, wie sie durch den Gebrauch chemisch neutral oder alkalisch eingestellter Hautreinigungsmittel auf der menschlichen Hautoberfläche bedingt werden, direkt die Zahl der Propionibakterien nicht aber der Staphylokokken zu beeinflussen vermögen.

Introduction

Aerobic Gram-positive cocci in particular *Staphylococcus epidermidis* and anaerobic gram-positive rods constitute major components of the regular bacterial flora of the human skin surface (15). Coagulase-negative staphylococci that is in particular *Staphylococcus epidermidis* in fact forms the major component of one of the most important biotopes of human skin, i.e. the so-called arid biotope as to be found on the volar side of the forearm. The same applies to *Propionibacterium* species primarily consisting of *Propionibacterium acnes* with respect to the sebum-rich biotope represented by the forehead (5). According to early experimental studies the human skin surface is acidic (17), it varies, however, according to the particular site of the body (13). This is said to be of influence on the local bacterial flora (14). Facing these findings *Marchionini* has coined the term acid-mantle as early as in 1928 (17). This concept, however, has been subject to controversial debate ever since within the scientific community (9).

Most recently the concept has been re-evaluated experimentally under the aspect of the development of optimum skin cleansers. The major findings can be summarized as follows:

Upon prolonged repeated use of a slightly acidic syndet (pH 5.5) the skin surface pH remains in the 5.5 range while it increases by about 0.3 units upon the corresponding application of an alkaline soap of about pH 9.0. Higher pH-values are linked to higher counts of *Propionibacterium* species at the skin surface (12). To find out if these effects of the cleansing preparations on skin pH and microflora are due to the pH of the preparation itself or to other factors chemically identical syndet preparations of pH 5.5 and 8.5 were also evaluated following the same trial design and the results were essentially the same (11).

Although these data suggest that the pH-value of the milieu exterieur directly influences the quantity of bacteria on the human skin surface the role of the pH-value on bacterial growth needs further investigation by in vitro studies. Preliminary data based on batch culture experiments already available in fact suggest that there is a marked difference with respect to the specific growth rate of propionibacteria at pH 5.5 and pH 6.0, while this is not the case with staphylococci (10). Coagulase-negative staphylococci correspondingly showed no clear-cut dependence on the varying skin surface pH-values in the human volunteers (12).

From a microbiological standpoint continuous culture experiments using a chemostat seem to be particularly apt to get deeper insight into the relationship between pH and bacterial growth (2, 4). In the following, corresponding experiments will be described considering the growth of both *Staphylococcus epidermidis* and *Propionibacterium acnes* at various pH-values. *Staphylococcus aureus* is also included as it is a major component of the skin flora in patients suffering from atopic dermatitis (6), a type of eczema which in fact is a skin disease related to elevated skin surface pH-values (8).

Materials and Methods

Microorganisms

Staphylococcus epidermidis. Recent clinical isolate from human skin (470/88) identified by colony morphology, missing hemolysis on blood agar, Gram stain, negative plasma coagulase test, characteristic pattern of metabolism as to be judged by a ready-made test system (API-STAPH, API Bio Merieux GmbH, Nurtingen, D; Code number: 670 41 12).

Propionibacterium acnes. Recent clinical isolate from human skin (F21) identified by colony morphology, Gram stain, typical pattern of metabolism due to a ready-made test system (API 20A, Code number: 500 25 44). In general, culture of propionibacteria was performed under anaerobic conditions using Gas Pak Jars with Gas Pak Plus (BBL Microbiology Systems, Cockeysville, MD, USA) as generator kit.

Staphylococcus aureus. Recent clinical isolate from human skin (3305/88) identified by colony morphology, hemolysis on blood agar, Gram stain, positive plasma coagulase test, typical pattern of metabolism as to be judged from a ready-made test kit (API STAPH, Code number: 633 61 51).

Culture media

Staphylococci. Before culturing staphylcocci in broth they were subcultured on blood agar. This medium contains

Blood agar base (Difco, Detroit, MI, USA)	40.0 g
defibrinated sheep blood	50.0 ml
destilled water	ad 1000.0 ml

In continuous culture, staphylococci were grown in Trypticase soy broth (Difco).

Propionibacteria. Before culturing propionibacteria in broth, they were subcultured on "TSY" agar. This medium contains

Trypticase soy broth (Difco)	30.0 g
Yeast extract (Difco)	10.0 ml
Tween® 80 (E. Merck, Darmstadt, D)	5.0 ml
Bacto agar (Difco)	20.0 g
destilled water	ad 1000.0 ml

In continuous culture, *Propionibacterium acnes* was grown in Clostridial reinforced medium (Oxoid Ltd., Basingstoke, Hampshire, England) (8).

Culture conditions

All bacteria were grown in a Biostat M chemostat (Braun Melsungen AG, Melsungen, D). In order to maintain a continous flow, a pump FE 411 (Braun Melsungen) was used. Growth conditions (temperature, rotation speed, pH-value, pO_2-value) were registered automatically by a multi-channel plotter Jumo Comp PD (Braun Melsungen).

With this system the following parameters can be addressed separately: Gas supply and flow of broth through the vessel can be pre-fixed as well as rotation speed while temperature, volume of broth in the vessel, addition of antifoam agent as well as the pH-value proper can be controlled.

Culturing staphylococci, the following parameters were chosen:

air flow: 0.2 l/min
temperature: 37 °C (+/− 0.1 °C)
rotation speed: 800 rpm
flow: 90 ml/h
volume of the culture vessel: 900 ml.

Samples were taken every 60 min during the first twelve hours. Afterwards samples were taken every twelve hours. In general, each experiment lasted 8 days. Culturing *Staphylococcus epidermidis*, each experiment was performed in duplicate.

Culturing *Propionibacterium acnes*, the following parameters were chosen:

nitrogen flow: 0.1 l/min
temperature: 37 °C (+/– 0.1 °C)
rotation speed: 800 rpm
flow: 18 ml/h
volume of the culture vessel: 900 ml.

Compared to the parameters for culturing staphylococci the flow rate of culture medium through the vessel was reduced. The flow rate must correspond to the specific growth rate of the cultured microorganism. Because of the low specific growth rate of propionibacteria compared to staphylococci the number of propionibacteria using a flow rate of 90 ml/h would be reduced by the flow rate itself (10).

To induce the pre-logarithmic growth phase of propionibacteria the experiments were started on a batch culture basis. This period lasted for 15 h after inoculation. During this period samples were taken at 0 and 15 h. Later on, samples were in general taken at 18, 21, 24, 27, 39, 42, 51 h and then every 24 h. Each experiment in general lasting 8 days was performed in duplicate.

As anti-foam substance "Silicon-Antischaum-Emulsion M-30 reinst" (Serva, Heidelberg, D.), diluted with destilled water 1 : 10, was used.

The pH-value was kept constant by the addition of 1M NaOH or 1M HCl.

To determine the bacterial density, i. e. the number of colony-forming units (CFU) per ml defined samples of liquid medium were diluted repeatedly (by the factor 1 : 10) and inoculated on blood agar and "TSY" agar, respectively.

The specific growth rate was determined by drawing a compensation line during the period of maximum growth (comprising the first 12 h with staphylococci, the period between 18 and 42 h after the start of the experiment with the slower growing propionibacteria which also exhibited a higher lagtime). As the growth kinetics of propionibacteria differed the period stated above had to differ, too. With the controlled pH-value of 7.5 the compensation lines were drawn between 42 and 66 h. The specific growth rate was obtained as the reciprocal value of the time required for doubling bacterial density.

Results

Staphylococcus epidermidis

With *Staphylococcus epidermidis* bacterial density lay between $10^{6.5}$ and $10^{7.0}$ at the beginning of the experiments (Fig. 1 and Fig. 2). Bacterial growth began without delay at pH-values of 5.5 and 7.0 (Fig. 1). Bacterial density was slightly reduced in both experiments at pH 8.5 during the first 12 h. The specific growth rates are given in Table 1. In so far as the experiments at pH 8.5 are concerned not only the initial phase of growth was taken into consideration to determine a specific growth rate but also the period between 48 and 60 h. This is based on additional 2-hourly measurements within this particular period during the performance of the experiment showing greater initial reduction of bacterial density. The data on bacterial density during the plateau phase as to be expressed by lg CFU/ml are given in Table 2.

Propionibacterium acnes

With *Propionibacterium acnes* bacterial density ranged from 10[illegible] to 10[illegible] at the beginning of the experiments (Fig. 3 and Fig. 4). As the logarithmic growth phase never

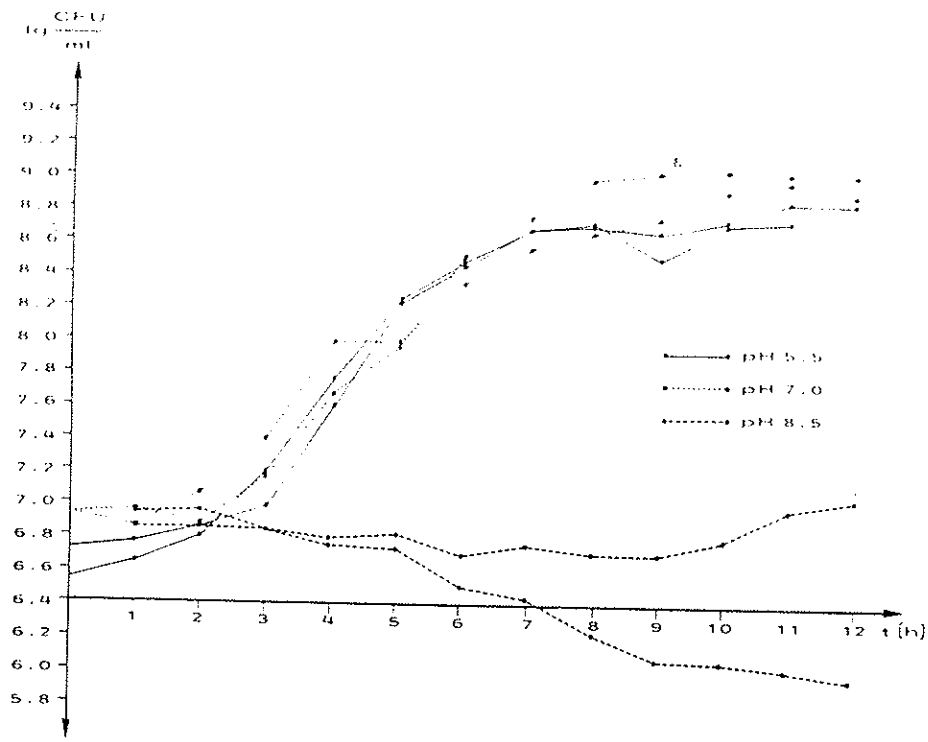

Fig. 1. Density of *Staphylococcus epidermidis* in continuous culture at differing pH-values in the short term.

Abb. 1. Dichte von *Staphylococcus epidermidis* in kontinuierlicher Kultur bei unterschiedlichen pH-Werten in der kurzen Frist.

began during the first 18 h of the experiment the specific growth rate was calculated on the basis of the data obtained during the consecutive 24 h (Fig. 5). As the growth curves with pH 7.5 were not parallel earlier than at 42 h the compensation line in this case links data obtained between 42 and 67 h. The specific growth rates are given in Table 1. With the pH-values of 6.0, 6.5, 7.0 a clear-cut plateau was seen (Table 2). With pH 5.0 and pH 7.5 no definite plateau could be detected. By and large the same applies to pH 5.5.

Staphylococcus aureus

With *Staphylococcus aureus* bacterial density ranged from $10^{7.1}$ to $10^{7.5}$ at the beginning of the experiments (Fig. 6 and Fig. 7). Bacteria grew nearly without delay at pH-values of 5.5 and 7.0 (Fig. 6). During the first 6 h, bacterial density was reduced by the factor 1000 at a pH of 8.5 (Fig. 6). Nevertheless, bacterial growth was as high as at

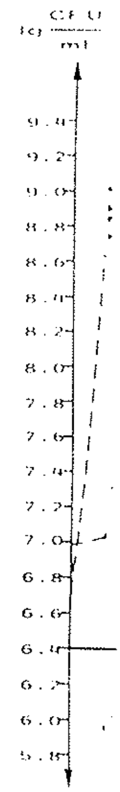

Fig. 2. Density of S...
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P. acnes
S. aureus

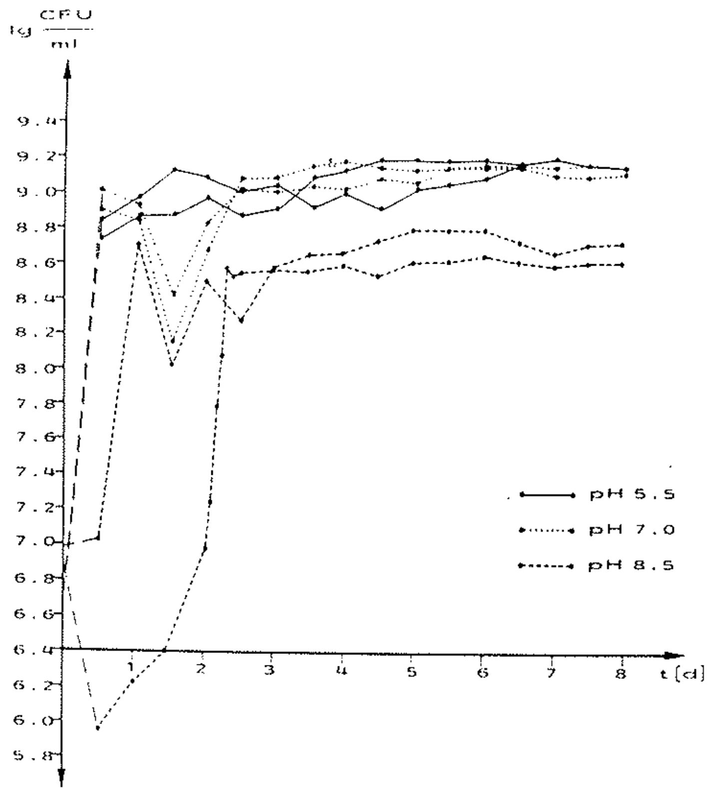


Fig. 2. Density of *Staphylococcus epidermidis* in continuous culture at differing pH-values in the long term.

Abb. 2. Dichte von *Staphylococcus epidermidis* in kontinuerlicher Kultur bei unterschiedlichen pH-Werten in der langen Frist.

Table 1. Specific growth rates (h^{-1}) of *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Staphylococcus aureus* in continuous culture. (Data in brackets represent the terminal specific growth rate while the other represent the initial specific growth rate; m.v.: missing value)

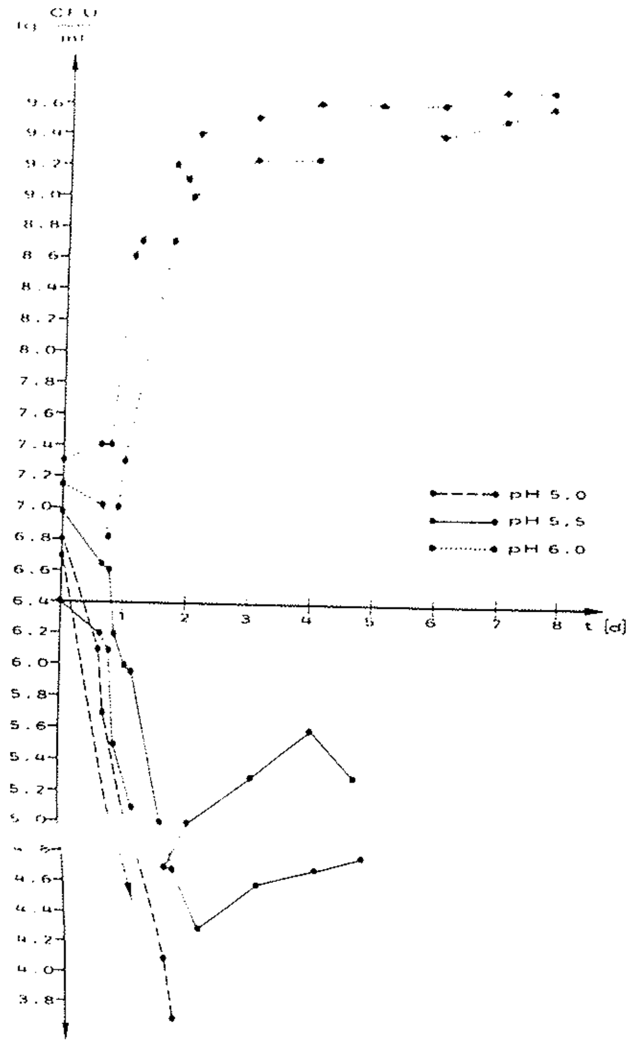
Tabelle 1. Spezifische Wachstumrate von *Staphylococcus epidermidis*, *Propionibacterium acnes* und *Staphylococcus aureus* in kontinuerlicher Kultur

	pH						
	5.0	5.5	6.0	6.5	7.0	7.5	8.5
S. epidermidis	–	1.71	–	–	1.08	–	–0.41 (0.68)
	–	1.66	–	–	1.21	–	–0.15
P. acnes	–0.28	–0.25	0.30	0.27	0.33	–0.13	–
	m.v.	–0.23	0.30	0.28	0.28	–0.12	–
S. aureus	–	1.40	–	–	1.19	–	–2.50 (1.33)

Table 2. Bacterial density (lg CFU/ml) of *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Staphylococcus aureus* in continuous culture during the plateau phase (m.v.: missing value

Tabelle 2. Bakteriendichte (lg KbE/ml) von *Staphylococcus epidermidis*, *Propionibacterium acnes* und *Staphylococcus aureus* in kontinuierlicher Kultur während der Plateau-Phase

	pH						
	5.0	5.5	6.0	6.5	7.0	7.5	8.5
S. epidermidis	–	9.1	–	–	9.1	–	8.6
P. acnes	m.v.	m.v.	9.6	9.5	9.2	m.v.	–
S. aureus	–	9.0	–	–	8.9	–	8.6



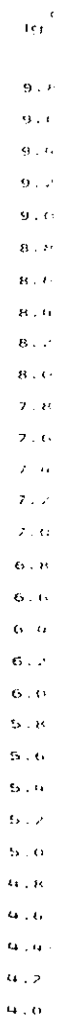


Fig. 4. Density of
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Fig. 3. Density of
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7.5	8.5
–	8.6
m.v.	–
–	8.6

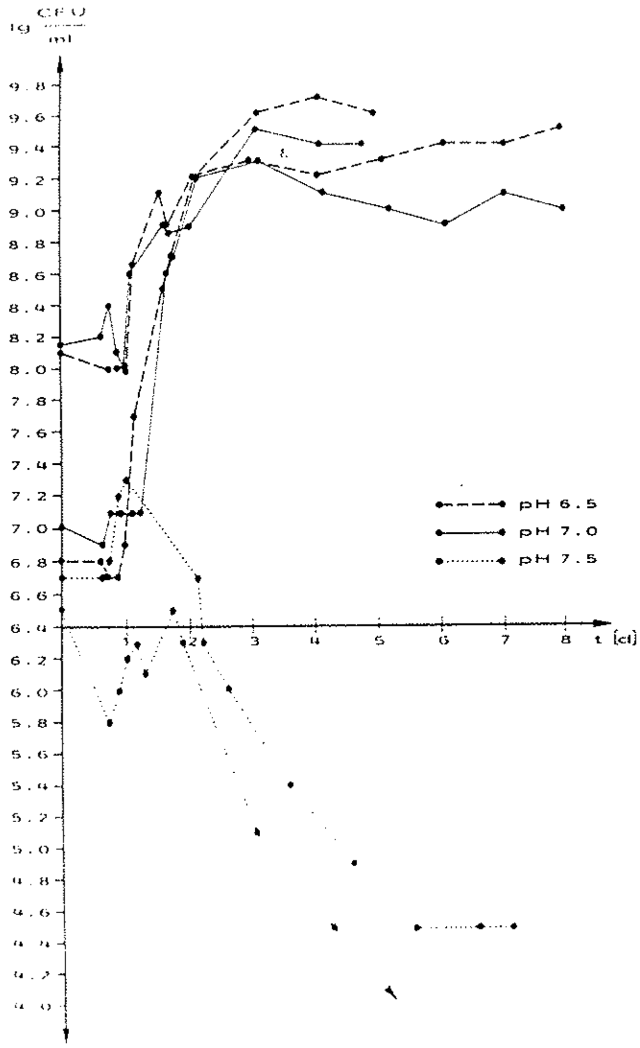
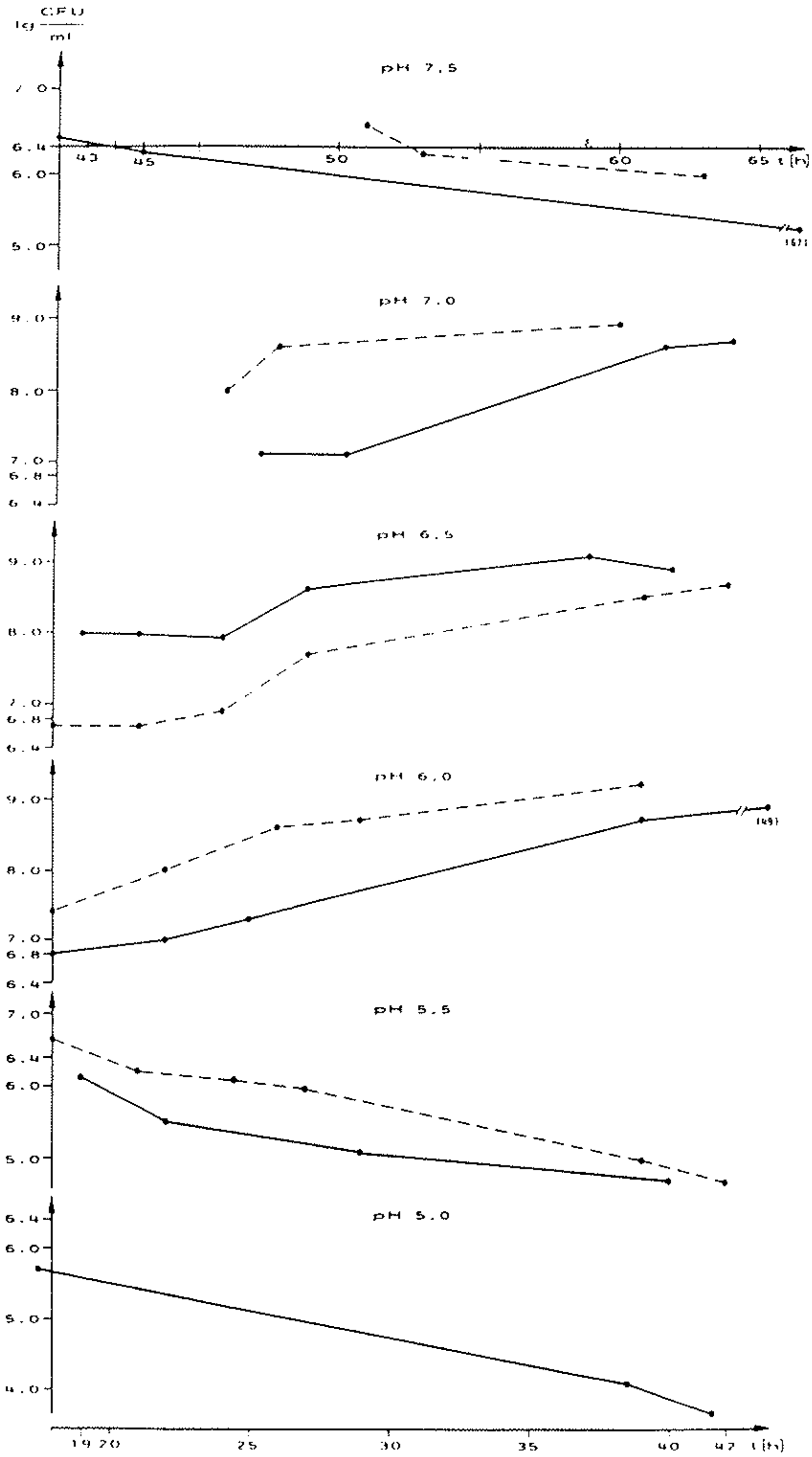


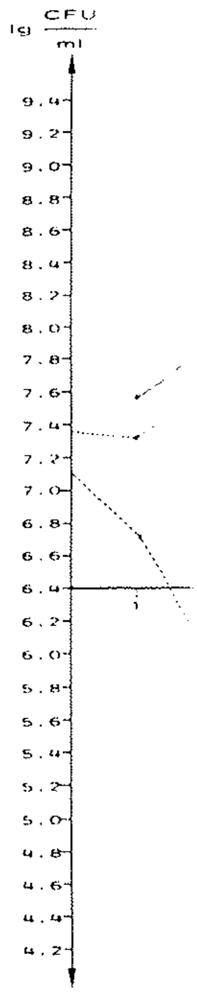
Fig. 4. Density of *Propionibacterium acnes* in continuous culture at differing pH-values in the long term (pH 6.5 to 7.5).

Abb. 4. Dichte von *Propionibacterium acnes* in kontinuierlicher Kultur bei unterschiedlichen pH-Werten in der langen Frist (pH 6.5 bis 7.5).

Fig. 3. Density of *Propionibacterium acnes* in continuous culture at differing pH-values in the long term (pH 5.0 to 6.0).

Abb. 3. Dichte von *Propionibacterium acnes* in kontinuierlicher Kultur bei unterschiedlichen pH-Werten in der langen Frist (pH 5.0 bis 6.0).





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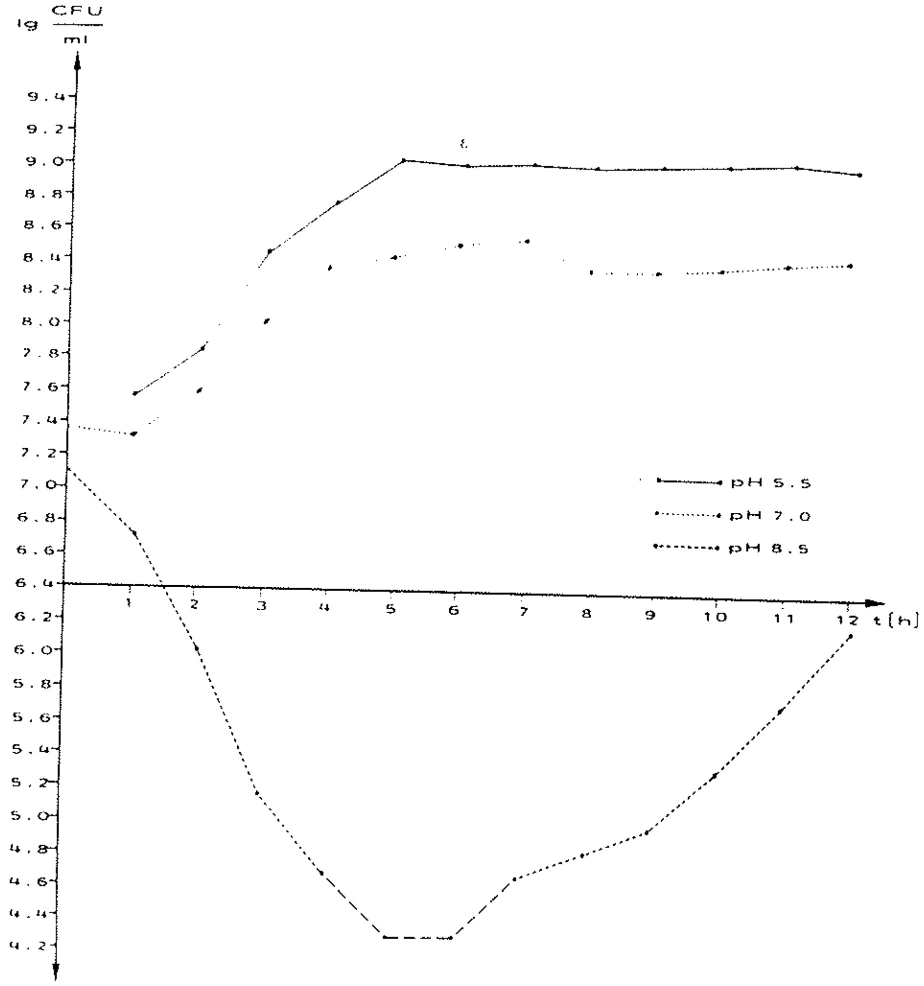

Fig. 6. Density of *Staphylococcus aureus* in continuous culture at differing pH-values in the short term.

Abb. 6. Dichte von *Staphylococcus aureus* in kontinuierlicher Kultur bei unterschiedlichen pH-Werten in der kurzen Frist.

Fig. 5. Density of *Propionibacterium acnes* in continuous culture at differing pH-values in the short term during 18 and 42 h with pH 5.0 to pH 7.0 and 42 to 67 h with pH 7.5 (neighbouring curves marked differently represent experiments performed in duplicate).

Abb. 5. Dichte von *Propionibacterium acnes* in kontinuierlicher Kultur bei unterschiedlichen pH-Werten in der kurzen Frist während Stunde 18 und 42 bei pH 5.0 bis 7.0 und 42 bis 67 bei pH 7.5.

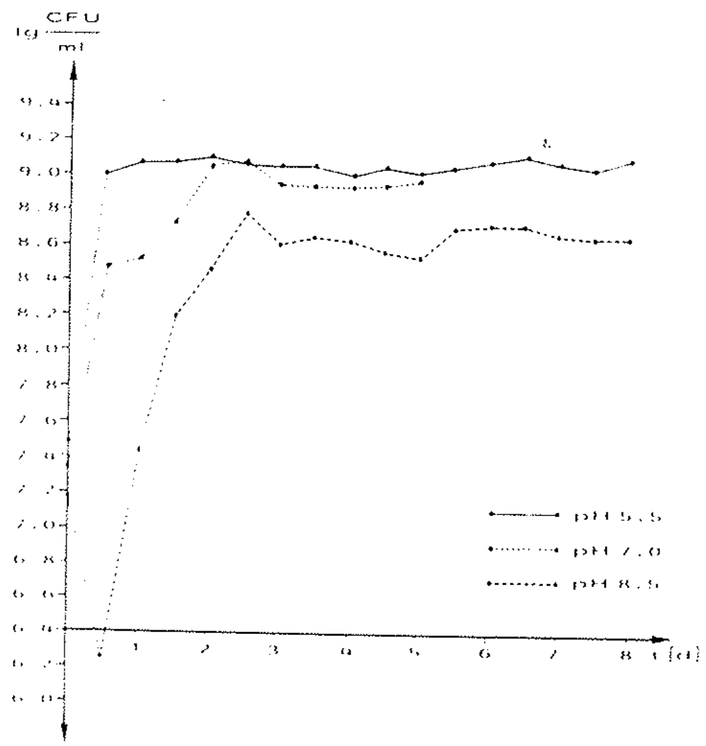

Fig. 7. Density of *Staphylococcus aureus* in continuous culture at differing pH-values in the long term.

Abb. 7. Dichte von *Staphylococcus aureus* in kontinuierlicher Kultur bei unterschiedlichen pH-Werten in der langen Frist.

pH-values of 5.5 and 7.0 between 6 and 12 h as can be derived from the ascent of the growth curves. Resulting specific growth rates are shown in Table 1.

Growth curves show a plateau with all pH-values (Fig. 7). The number of CFU/ml during the plateau phase is stated in Table 2.

Discussion

Both the growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* is linked to the pH of the milieu exterieur as to be judged from the present experiments in continuous culture. This confirms earlier findings in batch culture with *Staphylococcus aureus* (10). Yet there is no major difference between these two species in terms of the specific growth rate and the density during the plateau phase, in particular not with pH 5.0 and pH 7.0. This is in clear contrast to the findings with *Propionibacterium acnes*. With *Propionibacterium acnes* there is a major difference with respect to the specific growth rate this parameter being negative at pH 5.5 and positive at 6.0. Correspondingly, a clear-cut plateau phase is not even reached at pH 5.5 while this is the case with pH 6.0. This again corresponds well to the findings with the previous batch culture experiments (10). Hence, chemostat experiments allowing the determination of the growth of these bacterial species in continuous culture at varying defined pH-values substantiate the hypothesis based on long-term experiments addressing the influence of skin cleansing preparations of differing pH on the pH-value of the human skin surface as well as its bacterial flora (12).

In more detail, it is obvious that changes in the pH-value of the human skin surface as to be induced in practice by skin cleansers of varying pH values cannot alter the colonization by staphylococci to any major extent. This by principle applies both to *Staphylococcus epidermidis* and *Staphylococcus aureus* which implies that it cannot primarily be due to the slightly altered pH-value that *Staphylococcus aureus* can very often be found in high numbers on lesional (1) and non-lesional skin (18) of patients suffering from eczematous states. The fact that *Staphylococcus aureus* as well as *Staphylococcus epidermidis* do not grow well at pH 8.5 should not be of relevance with respect to the composition of the skin flora as pH-values that high are probably only rarely obtained if at all.

In terms of the specific growth rate both *Staphylococcus aureus* and *Staphylococcus epidermidis* differ markedly from *Propionibacterium acnes* in so far as staphylococci grow well at a wide range of pH-values while with propionibacteria optimum growth only is to be found at pH 6.0 to pH 7.0. An in-depth interpretation of the differences in the specific growth rate at 5.5 and 6.0 has also to take into account that propionibacteria are primarily found within the infundibulum of the hair follicle which is said to be slightly more alkaline than glabrous skin (7).

With *Propionibacterium acnes* the present findings to a certain extent substantiate previous observations by Holland et al. (8) finding a pH optimum for the growth of the organism at pH 6.0. These experiments, however, have been performed under conditions not defined in detail in the corresponding publication.

From a more general standpoint the results of our experiments demonstrate that continuous culture as made possible by using a chemostat provide us with the opportunity to analyze the complex mechanisms governing the composition of the human skin microflora. This by principle is in accordance with previous investigations addressing the role of the pH on colonic bacteria also based on continuous culture in vitro (3). To get more insight into the pathogenesis of acne vulgaris as an *Propionibacterium acnes*-associated disorder it might be of interest also to look into bacterial metabolism under such conditions. In this context, potential virulence factors such as enzymes exhibiting lipolytic activity (16) should be of particular interest.

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References

1. *Beare, J. M., E. A. Cheeseman, A. A. H. Gailey,* and *D. W. Neill:* The pH of the skin surface of children with seborrhoic dermatitis compared with unaffected children. Br. J. Dermatol. 70 (1958) 233–241
2. *Davison, B. H.* and *G. Stephanopoulos:* Effect of pH oscillations on a competing mixed culture. Biotechnol. Bioeng. 28 (1986) 1127–1137

3. *Edwards, C. A., W. I. Duerden,* and *N. W. Read:* The effects of pH on colonic bacteria grown in continuous culture. J. Med. Microbiol. 19 (1985) 169–180
4. *Finlayson, H. J.:* The effect of pH on the growth and metabolism of *Streptococcus bovis* in continuous culture. J. Appl. Bact. 61 (1986) 201–208
5. *Hartmann, A. A.:* Zusammensetzung der Hautflora. In: Hautreinigung mit Syndets, ed. by *O. Braun-Falco* and *H. C. Korting,* pp. 88–92. Springer, Berlin–Heidelberg–New York (1990)
6. *Hauser, C., B. Wuethrich, L. Matter, J. A. Wilhelm, W. Sonnabend,* and *K. Schopfer:* *Staphylococcus aureus* skin colonization in atopic dermatitis patients. Dermatologica 170 (1985) 35–39
7. *Holland, D. B.* and *W. J. Cunliffe:* Skin surface and open comedone pH in acne patients. Acta Derm. Venereol. 63 (1983) 155–158
8. *Holland, K. T., W. J. Cunliffe,* and *C. D. Roberts:* The role of bacteria in acne vulgaris: a new approach. Clin. Exp. Dermatol. 3 (1978) 253–257
9. *Korting, H. C.:* Das Säuremantelkonzept von *Marchionini* und die Beeinflussung der Resident-Flora der Haut durch Waschungen in Abhängigkeit vom pH-Wert. In: Hautreinigung mit Syndets, ed. by *O. Braun-Falco* and *H. C. Korting,* pp. 93–103. Springer, Berlin–Heidelberg–New York (1990)
10. *Korting, H. C., A. Bau,* and *P. Baldauf:* pH-Abhängigkeit des Wachstumsverhaltens von *Staphylococcus aureus* und *Propionibacterium acnes.* Ärztl. Kosmetol. 17 (1987) 41–53
11. *Korting, H. C., K. Greiner, K. Hübner,* and *G. Hamm:* Changes in skin pH and resident flora by washing with synthetic detergent preparations at pH 5.5 and 8.5. J. Soc. Cosm. Chem. 42 (1991) 147–158
12. *Korting, H. C., M. Kober, M. Mueller,* and *O. Braun-Falco:* Influence of repeated washings with soap and synthetic detergents on pH and resident flora of the skin of forehead and forearm. Acta Derm. Venereol. 67 (1987) 41–47
13. *Marchionini, A.* und *W. Hausknecht:* Säuremantel der Haut und Bakterienabwehr. I. Mitteilung. Die regionäre Verschiedenheit der Wasserstoffkonzentration der Hautoberfläche. Klin. Wschr. 17 (1938) 663–666
14. *Marchionini, A.* und *R. Schmidt:* Säuremantel der Haut und Bakterienabwehr. III. Mitteilung. Über die regionäre Verschiedenheit des Bakterienwachstums auf der Hautoberfläche. Klin. Wschr. 17 (1938) 773–775
15. *Mok, W. Y.* and *M. S. Barret da Silva:* Microflora of the human dermal surfaces. Can. J. Microbiol. 30 (1984) 1205–1209
16. *Reisner, R. M., D. Z. Silver, M. Puhvel,* and *T. H. Sternberg:* Lipolytic activity of *Corynebacterium acnes.* J. Invest. Dermatol. 51 (1968) 190–196
17. *Schade, H.* und *A. Marchionini:* Der Säuremantel der Haut (nach Gaskettenmessung). Klin. Wschr. 7 (1928) 12–14
18. *Schauwecker, R.:* Zur Frage der pH-Verhältnisse der nichtbefallenen Hautoberfläche bei Ekzematikern. Dermatologica 111 (1955) 197–203

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