Fermentative and Serological Studies on Propionibacterium acnes

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Seventy-two Propionibacterium acnes strains, among which were five from the American Type Culture Collection, five from the Center for Disease Control, and four of group II of Voss, were thoroughly examined both biochemically and serologically. On the basis of the fermentation of inositol, maltose, mannitol, and sorbitol, eight biotypes were distinguished. By means of tube agglutination tests with the five absorbed antisera, 95, C51, D34, S140, and Beck, 11 serotypes were defined. The biotypes and serotypes showed no striking relationship to each other. Combined biotyping and serotyping is suggested for subdivision of the P. acnes species.

Propionibacterium acnes, a gram-positive, nonmotile, nonsporulating and microaerophilic bacillus, is normally present on human skin. There exists little doubt about the pathogenic importance of P. acnes: this bacillus is not only regularly isolated from acne vulgaris lesions but very often also from actinomycosis and nonspecific anaerobic infections of humans. The taxonomic position of P. acnes is still controversial. Some authors associate this species with propionibacteria (5, 6, 11, 14), and others reject this proposal (20, 21). Identity of P. acnes with other species of anaerobic coryneforms is still under discussion (1, 3, 6, 10, 12, 17, 20, 21). Furthermore, there is considerable confusion about the characteristics of the P. acnes species itself and its subdivision. For these reasons, we began extensive studies on the differentiation of P. acnes.

MATERIALS AND METHODS

Strains. The 72 P. acnes strains used were obtained not only from the normal habitat but also from pathological human material. Among these strains were 14 sent from foreign centers for comparative purposes: strains CDC 5159, 6994, 6949, 6981, and 7010 from V. R. Dowell, Jr., Center for Disease Control, Atlanta, Ga.; strains ATCC 6919, 6921, 6922, 6925, and 11827 from the American Type Culture Collection; and strains D 34, C 51, D 21, and V 1 of his group II from J. G. Voss, The Proctor & Gamble Co., Cincinnati, Ohio. An additional 58 strains were isolated in this laboratory: 27 strains were cultivated from the hair of healthy humans (25 women and 2 men, strains H, to H31), and 31 were from human infectious processes (7 from acne vulgaris, 1 from actinomycosis, and 23 from nonspecific anaerobic infections of the cervicofacial region).

Cultivation. Cultivation and purification of P. acnes strains were carried out in accordance with the recommendations of Lentze (9) on 4% glycerol agar by the Fortner method (cultivation on the surface of solid agar media, a third of the surface heavily inoculated with Serratia marcescens, and the plates closed with glass dishes and plasticine). After not more than 10 days of incubation at 37 C, the P. acnes strains grew as typical hemispherical yellowish-white to red pigmented colonies (diameter, 2 to 3 mm).

Media. The basal medium for biochemical and serological studies was as follows: Casitone, 10 g; Difco yeast extract, 10 g; KH₂PO₄, 4 g; MgSO₄, 7H₂O, 1 g; and distilled water, 1,000 ml. For solidification, 28 g of Difco agar was added. The medium was sterilized in an autoclave, the corresponding carbohydrate (Seitz-filtered) being added to a final concentration of 1% (w/v). Water blue (wasserblau, chinablue, soluble blue; a mixture of sulfonates of triphenyl-p-rosanilin and diphenyl-rosanilin; distinctive color change at pH 6.0) was used as the pH indicator (0.4 ml of a 1% solution/100 ml of medium). After adjustment of the pH to 7.4, 2-ml samples were poured into small tubes and again autoclaved at 100 C for two periods of 10 min.

Inoculum. One drop of bacterial suspension harvested from solid medium in phosphate buffer (KH₂PO₄, 1.45 g; Na₂HPO₄, 7.6 g; NaCl, 4.8 g; distilled water, 1,000 ml) was used routinely. The density of suspension was standardized to the turbidity of the McFarland nephelometer tube no. 5. All carbohydrate fermentation reactions were read after 7 days of incubation at 37 C. A distinctive change of color of the pH indicator was taken as a positive reaction. All tests were repeated at least once.

Test methods. Catalase activity was investigated by adding two drops of a 0.3% H₂O₂ solution to P.
acnes colonies were transferred onto a clean slide. Indole production was checked after 5 days of incubation at 37 C in Hettlinger broth by addition of Kovacs reagent. Nitrate reduction (basal medium plus 0.5% KNO₃) was ascertained after 5 days of incubation at 37 C by adding a mixture of sulfanilic, acetic acid, and α-naphthylamine. H₂S production in the basal medium was checked by means of lead acetate paper. In the gelatin test, the basal medium was mixed 4:1 with gelatin. For the litmus milk test, Difco medium (litmus milk dehydrated, control no. 448415) was used. The H₂S, gelatin, and litmus milk tests were finally evaluated after 3 weeks of incubation at 37 C. After 7 to 10 days at 37 C, hydrolysis of urea or esculin (0.5 and 0.1%, respectively, in basal medium) was examined by addition of phenolphthalein or 0.5% ferric citrate solution. All biochemical tests were carried out under microaerophilic conditions (immediate sealing of the inoculated tubes with Vaseline).

Preparation of antigen suspension. For immunization and for all serological tests, mass cultures were required. This was achieved on the surface of the solid basal medium (5 days of incubation at 37 C). The bacteria were harvested and suspended in phenolic (0.5%) saline. This suspension was homogenized for 5 min in an ultrasonic disintegrator (MSE, 500 w) and standardized to the density of a McFarland nephelometer tube no. 5.

Antiserum preparation. Rabbits were injected 12 times, at intervals of 3 days, with 0.5, 1.0, 1.5, 2.0, and 2.5 ml, and then constantly with 2.5 ml through the twelfth injection. P. acnes suspensions were given intravenously. Tubes containing antiserum; the sera used in the test. This was achieved on the surface of the solid basal medium (5 days of incubation at 37 C). The mixture was centrifuged at 4,000 x g for 20 min. Absorption usually had to be repeated two or three times until all cross-reactions disappeared.

Agglutination test. Unabsorbed or absorbed rabbit antiserum was first diluted 1:20 in phosphate buffer (see above). Two drops of the antigen suspension were added to 0.5 ml of each serum dilution. The tubes were shaken thoroughly, incubated for 24 hr at 37 C (first reading), and left for a further 24 hr at 22 C (final reading). Positive and negative controls were always carried out. The serum dilution in which a clear agglutination could be seen at the final reading was considered as positive.

Precipitation test. The Ouchterlony agar-gel diffusion test was carried out according to Voss (19). Pure antigen suspension as well as the two following antigen preparations were used.

(i) Acetone extract: 50-ml volumes of liquid basal medium were heavily inoculated (material from five agar plates) and incubated for 3 weeks at 37 C. After centrifugation for 30 min at 1,000 x g, two volumes of cold acetone were added to one volume of the clear supernatant fluid, and this mixture was refrigerated at 4 C overnight. Centrifugation for 20 min at 4,500 x g followed, and the sediment was dissolved in one volume of distilled water.

(ii) Alcohol extract: bacterial suspension was washed twice with physiological saline. After centrifugation at 3,000 x g for 20 min, the sediment was suspended in 5 ml of phenolic (0.5%) saline. Treatment in an autoclave (120 C) for 30 min and centrifugation at 3,000 x g for 20 min followed. The supernatant fluid was mixed with 96% ethanol in a ratio of 1:4. This mixture was refrigerated at 4 C for 24 hr, followed by centrifugation at 4,000 x g for 20 min and suspension of the sediment in 1 ml of distilled water. This suspension was once more mixed with 96% ethanol in a ratio of 1:4 and refrigerated for 24 hr at 4 C. After centrifugation at 4,000 x g for 20 min, the supernatant fluid was rejected; the sediment was dried and thereafter dissolved in 1 ml of distilled water.

Agar-gel diffusion method. Three slides were placed in a case (LKB Sweden, 6801 A) and covered with a layer of 10 ml of agar prepared by dissolving 1 g of Reinagel Behringwerke in 100 ml of a mixture of 75 ml of distilled water and 26 ml of Veronal buffer (LKB Veronal buffer; ionic strength, 0.1, pH 8.6); phenol (0.25 ml) was added to the agar as a preservative. The gel was allowed to harden, and then five holes were cut out with an LKB gel punch, type 6808 A. The central hole was filled with the undiluted antiserum and the peripheral holes were filled with appropriate antigen preparations. After 24 hr at 22 C, precipitation bands were observed.

RESULTS

Fermentation tests. Results of the biochemical tests are summarized in Table 1. The reactions were in most cases easy to read, and repeated tests gave the same results. Fermentation patterns of the 72 P. acnes strains appeared to be stable. According to the proposal of Voss (19), strains 95, C 51, V 1, S 140, D 21, and D 34 belong to his group II, and all the other strains examined belong to his group I. Strain S 140 differed remarkably from the other group II strains, in that it hydrolyzed gelatin and fermented arabinose.

As the results presented in Table 2 show, it was possible on the basis of fermentation reactions with inositol, maltose, mannitol, and sorbitol alone to subdivide the 72 P. acnes strains into eight biotypes (biotypes A to H). Although a refinement of this differentiation system would be possible after consideration of reactions with arabinose, raffinose, gelatin, nitrate, and indole, this seems to be of little practical value.

Table 3 compares our results with those of other authors.

Agglutination tests. In preliminary experiments, the agglutination reactions proved to be quite useful in differentiation of P. acnes.
Therefore, it seemed worthwhile to determine the antigen spectrum of *P. acnes* by means of agglutination tests. Rabbit antisera agglutinating all 72 *P. acnes* strains were produced.

Absorption of antisera was necessary for exclusion of cross-reactions. The agglutination spectrum of five antisera, before and after absorption, can be seen in Table 4. These five antisera were most suitable also for serotyping of *P. acnes*.

Production of monospecific *P. acnes* antisera by reciprocal absorption was possible (Table 4). The necessary absorptions proved to be practicable; only two antisera (C 51 and D 34) sometimes caused difficulties, in that multiple absorptions led to a considerable decrease of potency.

All strains were thoroughly examined with the five absorbed antisera, and results are summarized in Table 5.

All 72 *P. acnes* strains tested reacted with at least one of the five antisera: 11 strains agglutinated with a single antiserum; 27 strains showed two, 22 strains three, and 12 showed 4; and 2 strains five, agglutination reactions, respectively.
### Table 3. Comparison of fermentative capabilities of Propionibacterium acnes found by different investigators

| Test          | Douglas & Guinter, 1946 | King & Meyer, 1957 | Brazin et al., 1967 | Moss et al., 1967 | Zierdt et al., 1968 | Ray & Kellum, 1970 | Present results |
|---------------|-------------------------|--------------------|---------------------|------------------|---------------------|-------------------|-----------------|
| No. of strains tested | 15                      | 37                 | 4                   | 15               | 29                  | 9                 | 38              |
| Adonitol      | 4/5*                    | 9/15               | -                   | -                | -                   | -                 | 31/38           |
| Arabinose     | -                       | 4/15               | -                   | +                | +                   | +                 | -               |
| Cellulbiose   | 6/15                    | 8/15               | -                   | -                | -                   | -                 | -               |
| Dextrin       | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Dulcitol      | -                       | -                  | 8/15                | -                | -                   | -                 | -               |
| Erythritol    | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Esculin       | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Galactose     | +                       | 36/37              | +                   | +                | +                   | +                 | +               |
| Glucose       | +                       | +                  | +                   | +                | +                   | +                 | -               |
| Glycerol      | 14/15                   | 34/37              | +                   | +                | +                   | +                 | +               |
| Glycogen      | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Inositol      | -                       | 2/15               | -                   | -                | -                   | -                 | 6/72            |
| Inulin        | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Lactose       | 3/5                     | 1/15               | -                   | -                | 4/61                | -                 | -               |
| Levallosul    | +                       | +                  | +                   | +                | 5/60                | +                 | -               |
| Maltose       | 11/15                   | 13/15              | 1/15                | -                | 4/61                | -                 | 29/38           |
| Mannitol      | 8/15                    | 14/29              | 6/37                | -                | 10/61               | -                 | 15/17           |
| Mannose       | +                       | +                  | +                   | +                | +                   | +                 | +               |
| Melibiose     | -                       | 2/15               | -                   | -                | -                   | -                 | -               |
| Melitizose    | -                       | 7/15               | -                   | -                | -                   | -                 | -               |
| Raffinose     | 6/15                    | +                  | +                   | +                | 4/61                | +                 | 6/72            |
| Rhamnose      | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Salicin       | -                       | 13/15              | -                   | -                | -                   | -                 | 6/72            |
| Sorbitol      | 4/5                     | 7/15               | -                   | -                | 21/38               | -                 | 6/72            |
| Sorbose       | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Starch        | 4/15                    | 5/15               | -                   | -                | -                   | -                 | -               |
| Sucrose       | 11/15                   | 7/15               | -                   | -                | 8/61                | -                 | 12/17           |
| Trehalose     | +                       | +                  | +                   | +                | 57/59               | -                 | 14/15           |
| Xylose        | -                       | 8/15               | -                   | -                | -                   | -                 | 6/72            |
| Nitrate       | +                       | +                  | +                   | +                | 29/38               | 54/61             | 8/11            |
| Indole        | 50%                     | 8/15               | 17/27               | +                | 34/38               | 48/61             | 119/129         |
| Gelatin       | -                       | -                  | -                   | -                | -                   | -                 | 6/72            |
| Urea          | -                       | -                  | -                   | -                | -                   | -                 | -               |
| H2S           | -                       | -                  | -                   | -                | -                   | -                 | -               |
| Litmus milk   | AC+                     | AC+                | AC+                | AC+              | AC+                | AC+              | AC+             |
| Methyl red    | +                       | +                  | +                   | +                | +                   | +                 | +               |
| Catalase      | +                       | +                  | +                   | +                | +                   | +                 | +               |

*Number of strains positive/total number of strains tested; + = all strains reacted positively; - = all strains reacted negatively; v = variable results; A = acid production; C = coagulation.

### Table 4. Agglutination spectrum of Propionibacterium acnes antisera before and after absorption

| Strain | Antisera | 95 | C51 | D34 | S140 | Beck |
|--------|----------|----|-----|-----|------|------|
|        | Unabsorbed |     | Unabsorbed |     | Unabsorbed |     |
| 95     | 1:640     | 1:640 | - | - | - | - |
| C51    | 1:160     | 1:160 | - | - | - | - |
| D34    | 1:640     | 1:320 | 1:640 | 1:80 | 1:40 | 1:80 |
| S140   | -         | -     | - | - | - | - |
| Beck   | 1:80      | 1:160 | 1:640 | - | 1:160 | 1:160 |

*Dash indicates no agglutination in antiserum diluted 1:20.
TABLE 5. Agglutination tests with 72 strains of Propionibacterium acnes with five absorbed antisera

| Sero-type | No. of strains | Strain no. | Absorbed antisera | 195 | C 51 | D 34 | S 140 | Beck |
|-----------|----------------|------------|-------------------|-----|-----|-----|-----|-----|
| 1         | 1              | 95         | 640*              | —   | —   | —   | —   | —   |
| 2         | 2              | C 51       | —                 | —   | 80  | —   | —   | —   |
|           |                | V 1        | —                 | —   | 80  | —   | —   | —   |
| 3         | 1              | D 34       | —                 | —   | —   | 80  | 20  | 80  |
| 4         | 1              | S 140      | —                 | —   | —   | —   | 160 | —   |
| 5         | 6              | Beck       | —                 | —   | —   | —   | —   | 160 |
|           |                | H 2        | —                 | —   | —   | 80  | —   | —   |
|           |                | ATCC 6923  | —                 | —   | —   | —   | 320 | —   |
|           |                | ATCC 6922  | —                 | —   | —   | —   | 80  | —   |
| 6         | 23             | H 1        | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 4        | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 5        | —                 | 20  | 320 | —   | —   | —   |
|           |                | H 6        | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 7        | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 9        | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 10       | —                 | 20  | 320 | —   | —   | —   |
|           |                | H 11       | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 13       | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 14       | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 15       | —                 | 20  | 320 | —   | —   | —   |
|           |                | H 16       | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 17       | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 18       | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 19       | —                 | 20  | 320 | —   | —   | —   |
|           |                | H 20       | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 21       | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 22       | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 23       | —                 | 20  | 160 | —   | —   | —   |
| 7         | 21             | H 24       | —                 | 40  | 640 | —   | —   | —   |
|           |                | H 25       | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 26       | —                 | 40  | 320 | —   | —   | —   |
|           |                | H 27       | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 28       | —                 | 40  | 320 | —   | —   | —   |
|           |                | H 29       | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 30       | —                 | 40  | 320 | —   | —   | —   |
|           |                | CDC 6981   | —                 | 40  | 20  | —   | —   | —   |
|           |                | CDC 6949   | —                 | 40  | 20  | —   | —   | —   |
|           |                | CDC 6923   | —                 | 40  | 20  | —   | —   | —   |
|           |                | CDC 7010   | —                 | 40  | 20  | —   | —   | —   |
|           |                | 3045       | —                 | 40  | 20  | —   | —   | —   |
|           |                | 172        | —                 | 40  | 20  | —   | —   | —   |
|           |                | 107        | —                 | 40  | 20  | —   | —   | —   |
|           |                | 112        | —                 | 40  | 20  | —   | —   | —   |
|           |                | 2630       | —                 | 40  | 20  | —   | —   | —   |
|           |                | 122        | —                 | 40  | 20  | —   | —   | —   |
|           |                | 2714       | —                 | 40  | 20  | —   | —   | —   |
|           |                | 902        | —                 | 40  | 160 | —   | —   | —   |
|           |                | 259        | —                 | 40  | 160 | —   | —   | —   |

*Figures are reciprocals of the highest antisem dilution at which agglutination occurred; — = no agglutination in antisem diluted 1:20.

TABLE 5—Continued

| Sero-type | No. of strains | Strain no. | Absorbed antisera | 195 | C 51 | D 34 | S 140 | Beck |
|-----------|----------------|------------|-------------------|-----|-----|-----|-----|-----|
| 8         | 3              | H 22       |                   | 20  | 160 | —   | —   | —   |
|           |                | 89         | —                 | 40  | 160 | —   | —   | —   |
|           |                | 4211       | —                 | 20  | 160 | —   | —   | —   |
| 9         | 1              | D 21       |                   | 80  | 80  | —   | —   | —   |
| 10        | 1              | ATCC 6919  |                   | 40  | 160 | —   | —   | —   |
| 11        | 12             | H 4        | —                 | 20  | 160 | —   | —   | —   |
|           |                | H 5        | —                 | 40  | 160 | —   | —   | —   |
|           |                | H 14       | —                 | 40  | 160 | —   | —   | —   |
|           |                | H 15       | —                 | 40  | 160 | —   | —   | —   |
|           |                | H 20       | —                 | 20  | 40  | 160 | —   | —   |
|           |                | H 21       | —                 | 20  | 40  | 160 | —   | —   |
|           |                | 79         | —                 | 40  | 40  | 160 | —   | —   |
|           |                | AK 1       | —                 | 40  | 20  | 160 | —   | —   |
|           |                | 147        | —                 | 40  | 20  | 160 | —   | —   |
|           |                | 2714       | —                 | 40  | 20  | 160 | —   | —   |
|           |                | Gerath     | —                 | 40  | 20  | 160 | —   | —   |
|           |                | 95         | —                 | 40  | 20  | 160 | —   | —   |

even four distinctive reactions. Altogether, 11 different reaction patterns were noted (serotypes 1 to 11). With regard to the grouping proposal of Voss (19), the following observations are noteworthy. Only the homologous P. acnes strain 95 (group II) reacted with the absorbed antisem 95. Of the 72 P. acnes strains examined, 41 were typable with one or both of the absorbed antisem C 51 and D 34 (both strains belonging to group II); 37 of these 41 strains were classified in group I. Fifty-six of the 66 group I strains but none of the group II strains could be agglutinated with the absorbed antisem S 140; the absorbed antisem Beck reacted with all 66 group I strains but with none of the 6 group II strains. By repeating these tests for control purposes, sufficient constancy was proved.

Heat resistance of the antigens was investigated. Five unabsorbed antisera were mixed with variously treated antigen suspensions of the five homologous strains. High heat resistance of the antigens was apparent: P. acnes cells which were heated for 30 min at 60 C, 30 min at 100 C, and even 60 min at 100 C showed reaction patterns which always corresponded to those of the untreated strains. The agglutination reactions actually appeared stronger after heat treatment. When bacterial suspensions treated for 60 min at 100 C were tested with the five absorbed antisera, the reaction spectra were the same as with untreated strains. It was clear that fractions of P. acnes which react as antigens possess high heat resistance, so that a protein nature of these antigens seems unlikely.

Precipitin tests. According to recommenda-
tions of Voss (19), antiserum D 34 (P. acnes strain of his group II) was absorbed with ATCC strain 6919 (his group I). As shown in Table 6, all P. acnes strains examined precipitated in unabsorbed antiserum D 34. With absorbed antiserum D 34, however, only strains of group II reacted. When whole broth culture was used as antigen, the reactions seemed to be weaker than with acetone or alcohol extracts. Of interest is the fact that strain S 140 belonging biochemically to Voss’s group II did not react with absorbed antiserum D 34. All further attempts to use the precipitin test for serological differentiation of P. acnes failed. Reactions with unabsorbed antisera 95, C 51, D 34, S 140, and Beck showed too many overlapping patterns, which sometimes differed depending on the antigen preparation. Mutual absorption failed to yield monovalent antisera which were potent enough for the agar-gel diffusion test.

**DISCUSSION**

The aim of these experiments was to clarify the confusion concerning the characteristics of P. acnes and to make an attempt to obtain reproducible biotyping and serotyping.

If one considers the published data concerning biochemical activities of P. acnes (see Table 3 and references 1, 18, and 20), it is noticeable that results achieved in recent years are comparable. In particular, our results correspond remarkably well with those of Voss (19). The differences we sought to clarify exist mainly in earlier reports.

For example, the assertion of Zierdt et al. (21) that P. acnes always ferments arabinose cannot possibly be correct. We found only a single arabinose-positive strain among 72 tested, and other scientists (7, 14, 15) found no strains fermenting this carbohydrate.

Also, the alleged acid formation from dextrin (2, 7), starch (2, 7), lactose (2, 7, 16), salicin (2), and xylose (2) must be judged with skepticism. These fermentation experiments were read only after unusually long incubation periods, i.e., up to 10 weeks. Data from corresponding investigations by other authors and our own results were clearly negative.

The trehalose fermentation of P. acnes, said to be always identifiable (2, 15, 16, 21), was not confirmed by Voss (19) or by us. Whereas Zierdt et al. (21) indicated the ATCC strains 6919, 6921, 6922, 6923, and 11827 as trehalose-positive, these strains were always trehalose-negative not only in the experiments of Voss (19) but also in our own. Although trehalose-fermenting P. acnes strains do exist (see Table

**Table 6. Precipitin tests with unabsorbed and absorbed Propionibacterium acnes antiserum D 34**

| P. acnes strain | Unabsorbed | Absorbed with ATCC 6919 |
|-----------------|------------|------------------------|
|                 | B | A | E | B | A | E |
| D 34            | + | + | (+) | + | + |
| 95              | + | + | (+) | + | + |
| C 51            | + | + | (+) | + | + |
| S 140           | + | + | - | + | - |
| ATCC 6919       | + | + | - | + | - |
| ATCC 6921       | + | + | - | + | - |
| ATCC 6922       | + | + | - | + | - |
| ATCC 6923       | + | + | - | + | - |
| ATCC 11827      | + | + | - | + | - |
| Beck            | + | + | - | + | - |

*Symbols: + = strong precipitation band with undiluted antisera; (+) = weak precipitation band with undiluted antisera; - = no precipitation band with undiluted antisera; B = whole broth culture used as antigen; A = acetone extract used as antigen; E = alcohol extract used as antigen.*

1), these strains are relatively rare and belong to group II of Voss (19).

Likewise, we found strains not liquefying gelatin almost exclusively in strains of group II of Voss (19; see Table 1). Statements of Ketron (7) and King and Meyer (8) must be therefore doubted. Ketron (7) emphasized that, in the gelatin culture medium he used, P. acnes strains grew very poorly.

Puhvel (15) found no acidification of litmus milk, which also conflicts with the results of other authors (5, 8, 14, 16) or our data. Puhvel (15) furthermore stated that the ATCC strains 6921, 6922, and 11827 do not reduce nitrate. Moss et al. (14) and we have proved, however, that there is always a definite reduction of nitrate by these strains. H2S production by P. acnes strains is not very pronounced (1, 14, 20).

By comparison of data shown in Table 3, the following typical fermentation spectrum for P. acnes can be offered, with the help of which a species differentiation should no longer be difficult: catalase reaction always positive; regular acid formation from galactose, glucose, glycerol, levulose, and mannose; dextrin, ducit, glycinogen, inulin, lactose, rhamnose, salicin, sorbose, starch, and xylose are not acidified; esculin and urea are not hydrolyzed. All other fermentation reactions show variations from strain to strain and are therefore not suitable for the species differentiation of P. acnes.

A subdivision of the P. acnes species has already been attempted by several authors
with varying success. Predominantly on the basis of morphological differences, Ketron (7) in 1927 established groups A and B. In 1942, Craddock (4) distinguished serologically different types I and II. In 1946, Douglas and Gunter (5) differentiated their 37 strains into four groups according to the varying fermentation of galactose, glycerol, mannitol, and maltose. They were of the opinion, however, that these variations would not be sufficient for a species subdivision of *P. acnes*. Brzin (2) found a saccharolytic and an indologenic type among 15 *P. acnes* strains which she tested. Her observations that maltose, sucrose, and melezitose are acidified only by indole-negative *P. acnes* strains, and vice versa, were confirmed both by Voss (19) and by us. Other serological tests (3, 8, 9, 11, 15), as well as exhaustive analysis of the fatty acids produced by *P. acnes* (13, 14, 16, 19), brought only negative evidence. In 1968, Zierdt et al. (21) tested their phage 174 on 167 *P. acnes* strains: 20 of these strains were phage-resistant, and among these least 9 strains showed still further deviation from the normal spectrum of characteristics.

Smith and Bodily (18) attempted to differentiate 50 diphtheroids: 9 strains of a group A were claimed to be *P. acnes*, 16 further strains of a group B were designated *P. acnes*-like strains, and the remaining 25 strains of the group C did not belong to the species *P. acnes*. We believe that 13 of the 16 strains of their group B can be classified with certainty and 3 further strains with some probability as *P. acnes*. Of the nine strains of their group A, seven strains agglutinated with the *P. acnes* antiserum 554 and 605 and two agglutinated only with antiserum 554. Sixteen strains of group B, on the other hand, gave only negative agglutination results in both antiseras. Ray and Kel- lum (16) reported in 1970 that 31 of their 61 *P. acnes* strains reacted with both antiseras 554 and 605, 11 further strains reacted only with serum 554, and 19 reacted with serum 605 only.

In 1970, Voss (19) classified 146 *P. acnes* strains, on the basis of biochemical and serological differences, into 129 strains of group I and 17 strains of group II. Only strains of his group II precipitated with antiserum D 34 which had been absorbed with ATCC strain 6919 (group I); this was not the case, however, with selected strains of his group I. Furthermore, the group II strains always showed a negative gelatin and indole reaction, and only 2 of 15 strains reduced nitrate. The majority of group II strains were capable of fermenting maltose, sucrose, melezitose, and trehalose, whereas none of the tested group I strains exhibited this property. The gelatin, indole, and nitrate tests were almost always positive in group I strains. These statements by Voss (19) were confirmed by us (see Tables 1 and 6) and can be checked on the ATCC and group II strains investigated by both of us. The only exception is strain S 140 which biochemically behaved as a group II strain, but serologically belonged to Voss's group I (negative precipitation reaction with absorbed antiserum D 34). Interesting also are our agglutination results showing that Voss's group II strains C 51 and D 34 actually possess a differing spectrum of antigens.

During the present work, Johnson and Cummins (6) published very important and interesting data. They compared 80 strains of anaerobic coryneforms with 29 strains of classical propionibacteria and eight strains of *Arachnia propionica* using cell wall analysis, deoxyribonucleic acid base composition, and nucleotide sequence similarity. The anaerobic coryneforms showed at least three homology groups, which were named as *P. acnes*, *P. avidum*, and *P. granulosum*. The *P. acnes* group was further subdivided into types I and II by differences in cell wall composition (galactose present or not) and cell wall agglutination. The ATCC strains 6919, 6922, and 11827, examined in our study also, were all located in type I. If we understood it correctly, the species *P. avidum* and *P. granulosum* differed from *P. acnes* in cell wall agglutination tests and in the degree of homology of deoxyribonucleic acid. All strains examined from Voss's group II, of which the strains C 51, D 21, and V 1 were tested also by us, belonged to *P. granulosum*. We agree with their well-documented proposal to place the anaerobic coryneforms into the genus *Propionibacterium*.

The precipitation test used by Voss (19) and also the cell wall agglutination test of Johnson and Cummins (6) seem to us not to be practicable for epidemiological investigations on *P. acnes*. Our data show clearly that a biotyping and serotyping of *P. acnes* can be done. Eight biotypes could be defined on the basis of fermentation reactions with inositol, maltose, mannitol, and sorbitol (Table 2). Agglutination tests in tubes with the five absorbed antiserum 95, C 51, D 34, S 140, and Beck led to 11 serotypes (table 5). A further subdivision could be reached by taking into consideration both biotypes and serotypes (Table 7). In contrast, precipitin tests proved to be of only limited value for typing of *P. acnes*. The needed absorption yielded antisera which were not potent enough for use in the agar-gel diffusion
Table 7. Comparison of biotyping and serotyping of Propionibacterium acnes

| Biotype | No. of strains | Serotype |
|---------|----------------|----------|
| A       | 38             | 1 2 3 4 5 6 7 8 9 10 |
| B       | 4              | 1 2 3 4 |
| C       | 4              | 1 2 3 4 |
| D       | 4              | 1 2 3 4 |
| E       | 13             | 1 2 3 4 5 6 7 8 9 10 |
| F       | 2              | 1 2 |
| G       | 5              | 1 2 3 |
| H       | 2              | 1 2 |
| Total no. | 72          | 1 2 3 4 5 6 7 8 9 10 |

Test. Preliminary encouraging results let us hope that phage-typing of P. acnes can be done in the near future.

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