The Behavior of Uv-Induced Coagulase-Positive and Negative Mutants of Staphylococcus aureus*

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The role of coagulase in staphylococcal infections is uncertain. It has been demonstrated(1) that coagulase-negative hemolytic staphylococci when treated with coagulase and injected intracerebrally into mice exhibit a higher degree of virulence than the same organisms without treatment with coagulase. Other workers(2,3) have also found an association of coagulase with virulence. However, Kapral and co-workers(4–6) have induced a mutant, Staphylococcus aureus strain 18ZD, from the parent S. aureus strain 18Z by uv irradiation. They have found that this mutant, although it produces coagulase as its parent does, is unable to multiply in the organs of the reticuloendothelial system of mice and rabbits while its parent possesses this ability. This work, as it stands, as well as the study of Alami and Kelly(7), have raised questions regarding the role of coagulase in staphylococcal infections.

The present study indicates that the uv irradiation employed for the production of coagulase-positive and negative mutants of S. aureus has altered the physiology of the above-mentioned mutants and such alterations induced by mutagens should be considered in assessing the role of coagulase in staphylococcal infections.

MATERIALS AND METHODS

Cultures. Staphylococcus aureus strain 18Z and its avirulent coagulase positive mutant 18ZD was kindly supplied to us by Dr. Kapral: S. aureus E33, a virulent

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coagulase-positive strain, and its virulent coagulase-negative mutant E33V were obtained from Dr. Kelly. They were tested for glucose and mannitol utilization under anaerobic conditions according to the method of Baird-Parker(8) and for gelatin liquefaction, pigmentation, hemolysis, both free and bound coagulase, and phage and antibiotic susceptibility by accepted microbiological methods(9). These strains were kept in stock in lyophilized samples and on trypticase soy agar (Baltimore Biological Laboratories) slants in screw-capped tubes at 4° and transferred every 4 weeks. Each transfer was followed by the diagnostic assays indicated in Table 1 to ensure that no alteration occurred upon storage. The selections of staphylococcal strains was based on the nature of amplification of the observation, or point, that the experiment was designed to supply.

Studies on skin lesion production. Six-month-old, white New Zealand male rabbits weighing 2.7–3.5 kg were used. The animals were shaved on the left and right side and infected on one side with varying inoculum of S. aureus strain E33 which had been grown aerobically in tryptic soy broth for 18 hr at 37°, and on the other side with similar doses of S. aureus strain E33V which had been grown under identical conditions. The injected animals were observed for 1–2 weeks. An injection was scored as a lesion if a pustule, or a central necrotic area appeared at the site of injection. The number of viable staphylococci in the skin of infected rabbits was also determined at varying intervals after infection. The skin lesions or areas of induration were excised aseptically, minced with scissors, and homogenized with a motor-driven tissue grinder. Serial 10-fold dilutions of the thoroughly homogenized suspensions were made in sterile saline and appropriate samples were plated on tryptic soy agar (Difco).

Manometric studies. Cells for the Warburg experiments were harvested from cultures which were at the same logarithmic stage of the growth cycle and had been grown in tryptic soy broth at 37°. The cells were washed twice with distilled water, and a smooth suspension was prepared by manual shaking for 5 min in a 125-ml Erlenmeyer flask containing 30 glass beads each having a diameter of 5 mm. The suspension was adjusted with distilled water to 500 Klett units using filter No. 66 and contained 0.8–1.2 mg of cells ml dry weight. In a typical experiment, each Warburg flask contained 1 ml of substrate, 0.5 ml of cell sus-

| Strain | Mannitol (anaerobic) | Dextrose (anaerobic) | Coagulase | Hemolysis (human blood) | Phage pattern | Antibiogram |
|--------|---------------------|---------------------|-----------|-------------------------|---------------|-------------|
| 18Z    | +                   | +                   | +         | +                       | +             | 52/52A/80/81 | AM/AU/CHL/ER/ME/NE/NO/OL/OX/PE/TE |
| 18ZD   | +                   | +                   | +         | +                       | +             | 52/52A/80/81 | Ibid. |
| E33    | +                   | +                   | +         | –                       | –             | 53/79       | Ibid. |
| E33V   | –                   | +                   | –         | –                       | +             | None        | AM/CHL/ER/ME/NE/NO/OL/OX/PE |
pension, and 0.5 ml of 0.1 M potassium phosphate buffer, pH 7.0. Two-tenths milliliter of 40% KOH was placed in the central wells. Rates of oxygen uptake in air were determined by conventional manometric techniques at 37°. The $Q_{O_2}$ values corrected for endogenous respiration, have been calculated from data obtained during the first 60 min and are expressed as $\mu l O_2$ consumed mg dry weight of cells per hr.

The concentration of such chemically defined substrates as sorbitol, mannitol, and pyruvate was 0.25% in all cases. This amount of carbohydrate was adequate to produce an oxygen uptake directly proportional to the duration of the assay procedures.

**Aerobic growth studies.** Cells for growth studies were grown at 37° for 24 hr in 5.0 ml of tryptic soy broth from an inoculum of one loopful and harvested by centrifugation. A smooth suspension was prepared by mixing the cells in sterile saline containing approximately 20 size-3 glass beads with a Vortex Junior Mixer for 3 min. The cells were adjusted to a density of 10 Klett units using a Klett-Summerson photoelectric colorimeter equipped with a No. 42 filter. The suspension contained approximately $2.0 \times 10^7$ colony-forming units as determined by correlative plate counts. One milliliter of the above suspension was inoculated into 250-ml nephelometric flasks containing 50 ml of growth media. The above were incubated on a rotary shaker at 37°. Turbidity determinations were performed by use of a Klett-Summerson photoelectric colorimeter equipped with a No. 42 blue filter.

**Growth studies with sodium chloride.** Flasks containing 50 ml of tryptic soy broth made 7.5 to 16% with respect to NaCl, were inoculated with 1 ml of a bacterial suspension adjusted to 20 Klett units. These were then incubated statically and aerobically for 24 hr. After mixing well, 5 ml of the cell suspension was removed and washed twice with saline. The cells were resuspended in 5 ml of saline and cell-turbidity determinations made as described previously.

**Growth at varying initial pH values.** Flasks containing 50 ml of tryptic soy broth were adjusted with HCl and NaOH to pH values ranging between 5 and 8. These were inoculated as described in the sodium chloride growth studies. After aerobic incubation for 24 hr, 5.0 ml of the cell suspension was washed twice and resuspended in 5 ml of saline. Turbidity determinations were made from this suspension.

**Studies on the reticuloendothelial system.** Young adult Swiss male mice, weighing 18–21 g were used in these experiments. Each experiment involved 10 control and an equal number of experimental animals of matched weights and was repeated 6–10 times. Experimental mice were injected through one of the tail veins with an 0.25 ml suspension of *S. aureus* containing $8 \times 10^6$–$1 \times 10^7$ colony-forming units per ml. Organs to be cultured were removed from animals killed 1–3 days after infection. Livers, kidneys, and spleens were washed in sterile saline and ground in 10-ml Thomas Teflon pestle model tissue grinders. Serial dilutions were made in saline and the number of colony-forming units (CFU) were determined by counting the colonies grown in trypticase soy agar pour plates. All plates were incubated in triplicate for 96 hr at 37° under aerobic conditions.
Electron microscopy. Sixteen-hour-old staphylococci were detached with a rubber spatula from the surface of rabbit blood agar plates (Difco) fixed in 2% glutaraldehyde in sodium cacodylate buffer at pH 7.2 for 1 hr at 4°, postfixed in 1% OsO₄, dehydrated via an alcohol series, and embedded in Epon. Ultrathin sections were cut with an LKB ultramicrotome and were stained with uranyl acetate and lead hydroxide. The specimens were examined and photographed in an RCA EMU-3H electron microscope operating at 100 V.

Preparation of cells for uptake studies. Cells for the uptake of nutrient experiments were harvested from cultures at the mid-logarithmic phase of growth and were cultured in Difco Tryptic Soy broth at 37° on a rotary shaker. The cells were washed twice with saline and a smooth suspension prepared by manual shaking for 5 min in a 125-ml Erlenmeyer flask containing 30 glass beads. The dry weight of cell suspension was estimated turbidimetrically in a Klett–Summer son photoelectric colorimeter equipped with a No. 42 filter and by the use of previously prepared standard curves. Under these conditions 1 Klett unit was equivalent to 1.5 ± 0.2 μg of cells dry weight per ml.

Total uptake of 14C-alanine. Staphylococci were suspended in buffer at the indicated pH and cell concentrations and preincubated at 37° on a rotary shaker for 30 min. The cells were added to 0.1 μCi of uniformly labeled 14C-alanine in a final volume of 1 ml. After 15-min exposure at 24°, 0.5 ml was filtered on a Millipore filter (Millipore Corporation, New Bedford, Ma.—0.45-μ pore size), presaturated with 10⁻³ M of 12C-alanine and washed with 5 vol of cold buffer. The filters containing the bacteria were air dried and placed in 9 ml of scintillation fluid(10). Radioactivity of filters was assayed according to the method of Mans and Novelli(11) in a Packard Tri-Carb liquid scintillation spectrometer model 3320. The efficiency of counting for radioactive carbon of the liquid-scintillation spectrometer was approximately 80%.

Fractionation of staphylococci. Bacteria were suspended in the synthetic medium of Hancock and Park(12) and preincubated on a shaker at 37° for 30 min. A specified volume of cells was added to 0.1 μCi of uniformly labeled 14C-alanine or 13C-glucose. After the indicated times of exposure, the suspension was immediately centrifuged at 4° and washed with ice-cold sodium phosphate buffer 0.1 M, pH 7.0. Then, the staphylococci were fractionated according to the procedure of Park and Hancock(13). Whenever Millipore filtration was employed all filters were presaturated with the corresponding unlabeled compound. In addition, the synthetic medium was slightly modified by deleting the 12C–constituent (alanine or glucose) corresponding to the 14C–compound under investigation.

Each experiment was repeated five times and the mean value or range of variation of these five trials was recorded.

RESULTS

Physiological reactions of staphylococcal strains. As a prelude to studies on the activities of the parent and mutant staphylococcal strains the physiological char-
acteristics of *S. aureus* strain E33, E33V, 18Z, and 18ZD were determined. The diagnostic features of these strains are listed in Table 1. The indicated biochemical reactions of strain 18Z and 18ZD were identical. Both strains fermented mannitol and dextrose, produced free and bound coagulase, hemolyzed human blood, were lysed by bacteriophages 52, 52A, 80, and 81 and were susceptible to ampicillin (Am 2 μg), aureomycin (AU 5 μg), chloramphenicol (CHL 5 μg), erythromycin (ER 2 μg), methicillin (ME 5 μg), neomycin (NE 5 μg), novobiocin (NO 5 μg), oleandomycin (OL 5 μg), oxacillin (OX 1 μg), penicillin (PE 2 units), tetracycline (TE 5 μg). As indicated by Kapral and Li(4) strain 18Z was sensitive to phage 42B. Our set of phages, purchased from Bell Products and Company (South Orange, NJ) did not include phage 42B. The mutant strain E33V differed from its parent strain E33 in its resistance to staphylococcal bacteriophages and its inability to ferment mannitol or produce free and bound coagulase. However, strain E33V also varied from its parent strain in its ability to hemolyze human blood and its resistance to aureomycin and tetracycline. Furthermore, supernatant fractions of aerated cultures of strain E33V contained a viscous material which was not found in culture supernatant fractions of strain E33. Figure 1a indicates that E33V possesses a larger capsule than that found in strain E33. No morphological differences were found between strain 18Z and 18ZD.

**Studies on skin lesion production.** An attempt was made to assay the ability of the above-mentioned strains to induce in rabbits staphylococcal skin lesions which constitute the prototype of staphylococcal infections. The results of these studies are summarized in Tables 2 and 3. Table 2 indicated that when the virulence of strain E33 and E33V was assayed i.d. in rabbits rather than, as previously, i.v. in mice(7) and the two sides of the same animal were employed in the assessment of virulence, the coagulase-positive strain E33 was able to produce pustular necrotic lesions in over 50% of the infected sides of animals with a dose of 50 Klett units (Approximately 8 × 10⁶ CFU), while the coagulase-negative strain E33V failed to do so even when a dose of 350 Klett units was employed. Furthermore, as shown in Table 3, the mean viable staphylococcal cell count in excised skin lesions of rabbits infected with strain E33 exceeded the cell count for strain E33V during the entire period of observation and with all the infective dosages tested.

| Inoculum in Klett units | Percent of injections showing pus and necrosis in 1 week |
|-------------------------|--------------------------------------------------------|
|                         | Strain E33 | Strain E33V |
| 350                     | 100        | 0-20        |
| 250                     | 100        | 0-10        |
| 150                     | 80-100     | 0           |
| 100                     | 70-100     | 0           |
| 50                      | 60-80      | 0           |
| 25                      | 30-60      | 0           |
BEHAVIOR COAGULASE MUTANTS IN STAPH.

Fig. 1. Electron micrograph of a segmented *S. aureus* strain E33. Fixed in 2% glutaraldehyde and 1% O$_3$O$_2$, × 50,000.

| Infective dose: | Mean total viable cell count per lesion at various hours |
|-----------------|---------------------------------------------------------|
| Klett units     | 48 hr | 72 hr | 192 hr | 48 hr | 72 hr | 192 hr |
| 350             | $2.4 \times 10^7$ | $3.8 \times 10^7$ | $1.7 \times 10^8$ | $1.2 \times 10^8$ | $7.6 \times 10^7$ | $6.0 \times 10^8$ |
| 250             | $1.2 \times 10^7$ | $2.5 \times 10^7$ | $3.0 \times 10^8$ | $6.4 \times 10^7$ | $5.4 \times 10^8$ | $1.3 \times 10^9$ |
| 150             | $1.0 \times 10^7$ | $1.3 \times 10^7$ | $2.1 \times 10^8$ | $5.9 \times 10^7$ | $4.6 \times 10^8$ | $1.6 \times 10^9$ |
| 100             | $6.7 \times 10^6$ | $8.8 \times 10^6$ | $7.1 \times 10^7$ | $1.6 \times 10^8$ | $4.4 \times 10^8$ | $3.2 \times 10^9$ |
| 50              | $6.0 \times 10^6$ | $9.5 \times 10^6$ | $3.6 \times 10^7$ | $8.0 \times 10^6$ | $5.8 \times 10^7$ | $0$ |
Studies with an avirulent coagulase-positive strain of *S. aureus*. It has been shown by Kapral and coworkers(4–6) that a mutant strain 18ZD obtained by uv irradiation of the parent strain 18Z of *S. aureus*, when injected i.v. into rabbits, multiplied in the RES at a rate equal to that of the avirulent *S. albus*(5–6) even though 18ZD produced coagulase. Since one characteristic of a useful mutant is that its growth pattern be similar to its parent, the in vivo and in vitro growth of strain 18Z and 18ZD was compared and is indicated in Table 4 and Fig. 2. In both instances, strain 18Z grew more luxuriantly than strain 18ZD in the reticuloendothelial system as well as in tryptic soy broth. Similarly, strain 18Z generally grew to a greater density than strain 18ZD at the same sodium chloride concentration (Table 5). In contrast to the above strains the reported(7) virulent coagulase negative mutant E33V grew faster in tryptic soy broth and showed greater salt tolerance than its parent strain E33.
To determine the ability of strains 18Z and 18ZD to grow at varying pH levels, the tryptic soy broth medium was initially adjusted to varying hydrogen ion concentration. The results of these experiments are listed in Table 6. At pH values of 7 and 8, strain 18Z was able to grow to a greater density than 18ZD. In the more acidic range of pH 6.0, strain 18ZD exhibited a much greater tolerance and was able to grow more luxuriantly.

Oxygen uptake studies. The results of the manometric studies are given in Table 7. Strain 18Z respired more actively than did strain 18ZD when human serum, tryptic soy broth, sodium pyruvate, and mannitol were used as substrates. These results indicate that the uv-light irradiation employed to produce the mutant strain 18ZD has affected the overall metabolism of this strain.

### Table 4
GROWTH OF S. aureus STRAINS 18Z AND 18ZD IN RES

| Strain   | Infective dose in Klett units | Mean number CFU organ at various hours |
|----------|-------------------------------|--------------------------------------|
|          |                               | Kidney 24 | 72  | Liver 24 | 72  | Spleen 24 | 72  |
| 18Z      | 350                           | 4.9×10^6  | 9.8×10^6 | 4.0×10^6  | 2.3×10^6  | 2.0×10^6  | 3.6×10^6  |
| 18ZD     | 350                           | 0         | 0     | 5.0×10^6  | 3.9×10^6  | 5.0×10^6  | 8.5×10^2   |
| 18Z      | 200                           | 4.4×10^6  | 7.6×10^6 | 7.2×10^6  | 4.6×10^6  | 1.0×10^6  | 2.9×10^6  |
| 18ZD     | 200                           | 2.4×10^6  | 1.0×10^6 | 6.7×10^6  | 1.7×10^6  | 9.0×10^6  | 1.0×10^6  |
| 18Z      | 100                           | 3.6×10^6  | 7.5×10^6 | 5.2×10^6  | 4.2×10^6  | 2.0×10^6  | 1.25×10^6 |
| 18ZD     | 100                           | 3.0×10^6  | 0     | 1.3×10^6  | 1.8×10^6  | 1.3×10^6  | 6.3×10^2   |
| 18Z      | 50                            | 6.8×10^6  | 3.7×10^6 | 3.9×10^6  | 2.6×10^6  | 1.4×10^6  | 9.0×10^6  |
| 18ZD     | 50                            | 6.9×10^6  | 0     | 2.7×10^6  | 2.3×10^6  | 4.6×10^6  | 1.3×10^6  |

### Table 5
GROWTH OF S. aureus STRAIN 18Z AND 18ZD IN TRYPIC SOY BROTH WITH VARYING CONCENTRATIONS OF SODIUM CHLORIDE

| Conc of NaCl (°/o) | Klett reading at 24 hr |
|-------------------|-----------------------|
|                   | Strain 18Z | Strain 18ZD |
| 7.5               | 155        | 155         |
| 10.5              | 115        | 74          |
| 12.5              | 77         | 23          |
| 14                | 43         | 0           |
| 16                | 10         | 0           |

### Table 6
GROWTH OF S. aureus STRAIN 18Z AND STRAIN 18ZD IN TRYPIC SOY BROTH AT VARYING INITIAL HYDROGEN ION CONCENTRATION

| pH | Klett reading at 24 hr |
|----|-----------------------|
|    | Strain 18Z | Strain 18ZD |
| 8.0 | 305        | 242         |
| 7.0 | 320        | 234         |
| 6.0 | 5          | 208         |
| 5.0 | 4          | 10          |
Uptake of nutrients by the uv induced coagulase-positive and negative staphylococcus aureus. Strains E33, E33V, 18Z, and 18ZD were suspended in 0.1 M sodium phosphate buffer pH 7.0 at a cellular concentration of 65, 222, and 406 µg dry weight per ml. After 30 min of preincubation at 37° on a shaker, the cells were added to 0.01 µCi of 14C-alanine for 15 min. Total uptake of 14C-alanine was determined by assaying radioactivity within the cells isolated by Millipore filtration (Table 8). In conformity with the rapid growth and high respiratory activity, the rates of 14C-alanine uptake by strain E33V were much larger at all cell concentrations than those observed for strain E33. At 65 µg/ml E33V yielded 15580 cpm while E33 contained 5,950 cpm; at 222 µg/ml E33V registered 33,300 cpm while E33 produced 14,040 cpm (58% difference); and at a cell concentration of 406 µg/ml, E33V contained 46,340 cpm while E33 contained 16,770 cpm (64% difference).

Similarly the total amounts of radioactivity accumulated by the metabolically active strain 18Z were greater than those accumulated by the metabolically sluggish strain 18ZD. At a cell concentration of 65 µg/ml there was a 46% difference;
at 222 μg/ml, a 34% difference, and at 406 μg/ml, a 32% difference. Further experiments were performed at the cellular density of 222 μg/ml.

*Antibiotics and nutrient macromolecular incorporation.* Experiments were performed with penicillin, which inhibits the binding of the terminal alanyl group to the cell wall mucopeptide. The parent and mutant strains were suspended in the synthetic medium in the presence and absence of 165 units per ml of penicillin and incubated for 60 min on a rotary shaker at 37°. The bacteria were added to uniformly labeled ¹⁴C-alanine for 15 min, or uniformly labeled ¹⁴C-glucose for 2 min, centrifuged, washed, and fractionated. The action of penicillin on the residue fraction which contains the cell wall mucopeptide were recorded in Table 9. When E33V was exposed only to radioactive alanine the residue fraction con-

### TABLE 7

**Oxidation of Various Substrates by S. aureus Strain 18Z and 18ZD**

| Substrate | Strain 18Z | Strain 18ZD |
|-----------|------------|-------------|
| Tryptic   | 619 ± 152  | 266 ± 83    |
| Human serum | 280 ± 59  | 214 ± 35    |
| Pyruvate  | 144 ± 17   | 30 ± 6     |
| Mannitol  | 28 ± 2     | 22 ± 1     |
| Sorbitol  | 24 ± 1     | 18 ± 4     |

### TABLE 8

**Effect of Cell Concentration on the Uptake of ¹⁴C-Alanine**

| Strain | 65 (cpm/ml) | 222 (cpm/ml) | 406 (cpm/ml) |
|--------|-------------|--------------|--------------|
| E33V   | 15580       | 33300        | 46340        |
| E3     | 5950        | 14040        | 16770        |
| % difference | 62      | 58           | 64           |
| 18Z    | 55590       | 117600       | 162890       |
| 18ZD   | 30020       | 78000        | 110400       |
| % difference | 46      | 34           | 32           |

### TABLE 9

**Action of Penicillin on Incorporation of Alanine and Glucose into the Cell Wall Mucopeptide of Parent and Mutant Strains**

| Treatment   | E33V (cpm/ml) | E33 (cpm/ml) | 18Z (cpm/ml) | 18ZD (cpm/ml) |
|-------------|---------------|--------------|--------------|---------------|
| ³⁴C-alanine  |               |              |              |               |
| Control     | 106158        | 63104        | 61447        | 40969         |
| Penicillin-treated | 61572   | 7257         | 14925        | 3867          |
| % Inhibition | 42           | 88           | 76           | 91            |
| ³⁴C-glucose  |               |              |              |               |
| Control     | 8713          | 3350         | 8770         | 3734          |
| Penicillin-treated | 5033   | 2423         | 5072         | 2015          |
| % Inhibition | 12           | 28           | 42           | 46            |
tained 106,158 cpm, while in the presence of penicillin it registered 61,572 cpm. This corresponds to a 42% inhibition. The strain E33 residue fraction yielded 63,104 cpm and in the presence of penicillin it contained 7,257 cpm, corresponding to an 88% inhibition. When the above strains were exposed to $^{14}$C-glucose penicillin, entry of the label into the residue fraction was retarded by 12% for E33V and 28% for E33. Similar results were obtained for strains 18Z and 18ZD. Penicillin inhibited entrance of alanine into 18Z by 76% and 18ZD by 91%.

**DISCUSSION**

It has been shown by Kapral and co-workers(4-6) that the coagulase-positive mutant of *S. aureus* strain 18ZD when injected iv into experimental animals grew in the liver, spleen, and kidneys of the infected animals at a rate equal to that of the avirulent *S. albus*. The results reported in this investigation agree with those of Kapral and co-workers. However, one important characteristic of a useful mutant is that its growth pattern be similar to that of the wild type. The present *in vivo* and *in vitro* study indicated that the parent virulent strain 18Z grew more luxuriantly than the avirulent mutant 18ZD not only in the organs of the reticuloendothelial system but also in tryptic soy broth. This finding may possibly give some insight as to a cause for the observed differences in the virulence of strains 18Z and 18ZD. All other things being equal, an organism which is not able to multiply at a rate sufficient to establish itself in its host before the host can adequately mobilize its own defenses is not likely to be as successful as another which can multiply at a faster rate. *S. aureus* strain 18Z in tryptic soy broth has a lag time which is approximately one half that of strain 18ZD and the generation time of strain 18Z is almost 8 min shorter than that of 18ZD. Similarly, when manometric studies were performed, strain 18Z respired more actively than strain 18ZD when tryptic soy broth, serum, sodium pyruvate, and mannitol were used as substrates. A further indication for a general physiological difference between the two strains may be found in the results of the salt-tolerance experiments and the results of the growth responses at varying hydrogen ion concentration. On the other hand, if one considers the routine staphylococcal diagnostic tests for the parent and mutant strains, one would be impressed by the close similarity existing between these strains. However, it should be pointed out that assessment of a physiological activity, or the production of a toxin by routine diagnostic procedures does not yield a precise quantitative measurement of the physiological activity or the quantity of a given toxin, which is of germane importance in the outcome of an infectious process.

Regarding strains E33 and E33V, the data indicate that the reported(7) virulence of the coagulase-negative strain E33V depends on the experimental system. Thus, when the virulence of strain E33 and E33V was assayed intradermally in rabbits, rather than as previously intravenously in mice(7), and the two sides of the same animal were employed in the assessment of virulence, the mutant strain E33V was found to be weakly pathogenic as compared to its parent coagulase-
positive strain E33. To what extent the possession of a large capsule by strain E33V might have contributed to its virulence cannot be determined from this study. However, it has been pointed out by Koenig et al. (14,15) that staphylococci of the Smith and other encapsulated strains are more virulent for experimental animals than ordinary staphylococci.

Apart from the production of any particular extracellular substance, it seems reasonable to suggest that the altered activity of mutants to take up various nutrients will be added factors to be considered. Thus, before a meaningful delineation of the role of coagulase in the pathogenicity of S. aureus is to be made one must either discover a mutant which has fewer specifically and nonspecifically virulence-related differences, or precisely calculate the participation of each specific and nonspecific factor in the total virulence of each system used.

Though the other two mutants used by other investigators (4–6), one lacking bound coagulase and the other lacking soluble coagulase, were not tested in the present study, the same ideas presented here in regard to strain 18ZD may also have relevance to them. Before they are used for any comparison involving a single characteristic, their general physiological and metabolic characteristics should be delineated.

In previous studies (4–7), which questioned the role of coagulase in staphylococcal infection, no difference, other than the difference in virulence, was reported between the parent and mutant strains. The present investigation has elucidated some differences between the strains. These consist of various physiological and metabolic characteristics which could conceivably influence the virulence of the organisms studied.

**SUMMARY**

An important characteristic of a useful mutant is that its rate of growth be similar to that of the wild type. *In vivo* and *in vitro* experiments in this laboratory with a coagulase-positive mutant 18ZD of the virulent, coagulase-positive parent strain of *Staphylococcus aureus* 18Z indicate that strain 18Z grows more luxuriantly in tryptic soy broth, as well as in the various organs of the reticuloendothelial system than does strain 18ZD. When manometric assays were performed, strain 18Z respired more actively than strain 18ZD with human serum, tryptic soy broth, mannitol, and pyruvic acid as substrates. In conformity with the rapid growth and high respiratory activity, the rate of 14C-alanine, or 14C-glucose uptake by strain 18Z was significantly greater than that observed for strain 18ZD, both in the presence and absence of penicillin. These results indicate that the UV treatment used by Kapral and co-workers for the production of the mutant strain 18ZD has affected the overall growth pattern of this organism and may explain the reported observation that strain 18ZD multiplied in the RES at a rate approximately equal to that of the avirulent *S. albus* despite its ability to produce coagulase. Similarly, varying rates of growth, enhanced capsuleation, uptake of nutrients, and altered ability to induce experimental furuncu-
losis in rabbits were obtained for a previously reported virulent coagulase-negative mutant E33V and its parent strain E33 of S. aureus. Thus, these alterations induced by mutagens should be considered in assessing the role of coagulase in the evolution of staphylococcal infection.

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