Bactericidal effects of human sera versus pathogens

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Summary. Human sera, free of antibiotics, was found to possess varying degrees of bactericidal activity. A significantly greater amount of activity was seen against gram negative than gram positive bacteria.

The bactericidal (cidal) activity of antibiotic-free human sera against various gram negative bacteria has been previously reported[1-5]. These organisms were termed serum-sensitive. It was also noted[4] that serum-sensitive assay strains can provide misleading results in the Schlichter test[6] which is used to monitor antibiotic activity. This investigation compares innate serum cidal activity of numerous individuals against 12 commonly-isolated gram positive and negative pathogens at this institution.

Materials and methods. Fresh antibiotic-free sera was obtained from healthy laboratory personnel and infection-free patients. The sera were not heat inactivated and were tested within 24 h after collection. Serum bactericidal testing was by broth (Mueller-Hinton) microdilution. The inocula consisted of 10^5 organisms/ml. Final volumes in each microdilution plate well were 0.1 ml. Plates were incubated at 35 °C for 18 h. Bacteriostatic (static) levels were read as the greatest serum dilution without apparent growth. Broth (0.02 ml) from all wells without apparent growth was streaked onto a 5% sheep blood agar plate to detect viable organisms. The greatest dilution void of live organisms was taken as the serum bactericidal level.

Table 1. Serum cidal levels on laboratory personnel

Organism	Dilutions (reciprocal) Bacteriostatic Mean	Range	Bactericidal Mean	Range
Staphylococcus aureus ATCC 25923	0	0	0	0
Staphylococcus aureus No. 5757	0	0	0	0
Escherichia coli ATCC 25922	1/0.5	0-1	1/0.2	0-1
Escherichia coli (H)	1/4.5	1-1/8	1/2.4	0-1/8
Klebsiella pneumoniae ATCC 13883	1/2.2	1-1/4	1/2	0-1/4
Klebsiella pneumoniae No. 5360	1/0.8	0-1/2	1/0.2	0-1
Pseudomonas aeruginosa ATCC 27853	1/0.2	0-1	0	0
Pseudomonas aeruginosa No. 5904	1/2.8	1/2-1/8	1/0.9	0-1/8

Table 2. Serum cidal levels on hospital patients

Organism	Dilutions (reciprocal) Bacteriostatic Mean	Range	Bactericidal Mean	Range
Staphylococcus aureus ATCC 25923	0	0	0	0
Staphylococcus epidermidis ATCC 27626	1/1.5	0-1/2	0	0
Group D *Enterococcus*	0	0	0	0
Group A *Streptococcus* No. 7698	0	0	0	0
Escherichia coli (H)	1/2.6	1-1/8	1/2.2	0-1/8
Enterobacter agglomerans A17	1/1.7	0-1/2	1/0.6	0-1/2
Klebsiella pneumoniae ATCC 13883	1/3.6	1/2-1/8	1/2.4	0-1/4
Serratia marcescens No. 6945	1/1.7	1-1/2	1/1.3	0-1/2
Proteus mirabilis No. 7219	1/1.3	1-1/2	1/0.1	0-1/2
Proteus vulgaris No. 4767	1/1.9	1-1/4	1/0.3	0-1
Acinetobacter calcoaceticus var. *anitratus*	1/1.8	1-1/2	1/0.9	0-1/2
Pseudomonas aeruginosa No. 5904	1/4.9	1-1/8	1/3	0-1/8

Table 3. Serum cidal levels on three individuals

Organism	Dilutions (reciprocal) Bactericidal levels of patients: A	B	C
Staphylococcus aureus ATCC 25923	0	0	0
Staphylococcus epidermidis ATCC 27626	0	0	0
Group D *Enterococcus*	0	0	0
Group A *Streptococcus* No. 7698	0	0	0
Escherichia coli (H)	1/2	1	1/2
Enterobacter agglomerans A17	1	1	1/2
Klebsiella pneumoniae ATCC 13883	1/4	1	1/2
Serratia marcescens No. 6945	1/2	1	1
Proteus mirabilis No. 7219	0	0	1
Proteus vulgaris No. 4767	1	1	1
Acinetobacter calcoaceticus var. *anitratus*	1	1/2	1/2
Pseudomonas aeruginosa	1/2	1/8	1/2

Results. Table 1 summarizes the serum bacteriostatic and cidal levels of 13 laboratory workers against 2 strains of 4 different organisms. Levels varied from organism to organism and person to person. No activity was found against the 2 *Staphylococcus aureus* strains. This finding prompted the subsequent testing of 12 strains of *S. aureus* against sera from 21 patients. Again, absolutely no static or cidal activity was detected against the *S. aureus* strains. Table 2 summarizes serum static and cidal levels of 30 hospital patients vs 12 different organisms. The lack of cidal activity against gram positive organisms was evident. Activity vs gram negative organisms varied somewhat. There was no significant difference between serum cidal levels from laboratory personnel and hospital patients with the unexplainable exception of *Pseudomonas aeruginosa* No. 5904. Table 3 notes the differences in serum cidal activity of 3 patients against 12 bacteria. Various sera retested after 2–7 days storage at 4 °C lost significant amounts of activity. Inocula were found to be stable, regarding the number of viable organisms/ml, for 5 days when held at 4 °C.

Discussion. Various soluble humoral substances are apparently responsible for the bactericidal activity of serum. Among these substances are complement, properdin, lysozyme, phagocytin, antibodies against various bacteria, fatty acids, acid hydrolases, alkaline polypeptides and perhaps components of the myeloperoxidase system found in lysosomes. The extent to which each of these, and possibly other factors, contribute is unknown but likely varies among people and different factors may inhibit or kill different organisms. The common lack of post-infection immunity to staphylococcal infections may be related to the absence of serum cidal activity. In this study, serum from a given individual exhibited various activity against different organisms, suggesting that different mechanisms may be involved in the destruction of different organisms.

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Inhibition of *Physarum* mitochondrial division by cytochalasin B

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Summary. The mitochondrial division of *Physarum* is inhibited by cytochalasin B. Dumbbell-shaped dividing mitochondria become spherical bodies by this inhibitor. These results suggest that contractile proteins are essential for the mitochondrial division.

Physarum mitochondria contain an electron dense nucleoid, which consists of a large amount of DNA[2], RNA[3], and protein[2–4]. In the division of *Physarum* mitochondria, it is possible to observe 2 separate processes, the separation of the daughter mitochondrial nucleoids and mitochondriokinesis (mitochondrial division excluding nucleoidal division).

Materials and methods. Mitotically synchronized plasmodia of *Physarum polycephalum* were prepared by fusion of microplasmodia as reviewed by Guttes and Guttes[5]. Surface plasmodia between the 2nd postfusion mitosis (MII) and the 3rd postfusion mitosis (MIII) were used in these experiments. The 2nd synchronous nuclear division occurred 18 h after fusion, and the 3rd division at 28 h, while semi-synchronous mitochondrial division occurred within 5 h after the mitosis[6,7]. Cytochalasin B was employed according to the method of Axine and Peaven[8]. Small explants in diameter about 3 mm from the plasmodium at 1 h after MII were incubated for 3 h in a nutrient medium[5] containing 50 µg/ml cytochalasin B (Serva) or in control medium. These explants were harvested from 0–10 h later at intervals of 2 h after cessation of cytochalasin B treatment, fixed in Champy's solution[6] or in 6% glutaraldehyde and 1% OsO_4 solution[6] and prepared for light or electron microscopy by standard methods. An attempt to decorate actin filament with heavy meromyosin was done according to the procedures described previously[9]. Ultrathin sections were stained with both saturated uranyl acetate and lead citrate[6] and examined with a Hitachi-11E electron microscope.

Results and discussion. Compared with the mitochondrial division of higher eukaryotes, *Physarum* mitochondrial division is very simple. A spherical mitochondrion elongates, becomes ovoid and grows into a dumbbell-shaped mitochondrion (figure, A and B). The dumbbell-shaped mitochondrion divides semi-synchronously during mid S to form 2 spherical daughter mitochondria[10]. At that time, the dumbbell-shaped nucleoid also divides and separates into daughter mitochondria[6]. The dividing mitochondria of control preparations can be seen in plasmodia during late S fixed in Champy's solution (figure, A) or glutaraldehyde-OsO_4 solution (figure, B). When the ovoid mitochondrion elongates in a longitudinal direction and reaches a length of about 3.0 µm, a limiting membrane begins to invaginate in the equatorial region of the mitochondrion (figure, B). On the other hand, when a small explant of the plasmodium was exposed to cytochalasin B at 50 µg/ml for 3 h before fixation, a large number of mitochondria exhibited the large spherical or ovoid configuration (figure, C). The large spherical or ovoid mitochondria contain a V-shaped mitochondrial nucleoid (figure, D) or 2 nucleoids. Since dumbbell-shaped nucleoid in *Physarum* dividing mitochondrion was readily bent in the middle when isolated dumbbell-shaped mitochondrion was swelled and transformed into a spherical body[11], it appears that the large spherical or ovoid mitochondria containing V-shaped nucleoid may be in the division stage.

Cytochalasin B is well known as a specific inhibitor of the function of microfilaments[12]. Therefore, the simplest explanation for the absence of dumbbell-shaped mitochondria is that cytochalasin B has disrupted the function of contractile proteins so that the mitochondrion fails to become dumbbell-shaped. Our observations are consistent with the previous biochemical data which suggested that