INTRACELLULAR SURVIVAL OF STAPHYLOCOCCI*

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These studies on the intracellular survival of staphylococci were begun with the idea of investigating possible mechanisms of "cellular immunity" in staphylococcal infections. At that time the reports of Rogers and Tompsett (1) and Baker (2) had stated that virulent staphylococci would not only survive, but actually multiply within human and rat leucocytes. These reports prompted the present authors to see whether staphylococci could multiply within rabbit leucocytes and if this were the case whether rabbits "immunized" with staphylococci would exhibit an increase in cellular immunity such as has been reported to occur in tuberculosis and *Brucella* infections (3–5), in which cells from immune animals seem to suppress intracellular multiplication of the organisms. However, it soon became evident that "normal" rabbit and human leucocytes did not permit virulent staphylococci to multiply intracellularly. This suggested that the so called normal individual possessed a considerable degree of resistance to the organism without additional immunization. This report presents data to support the conclusion that virulent staphylococci only survive, but do not multiply within normal leucocytes of human and rabbit origin, while the organisms are actually destroyed by rat monocytes. These studies were performed both in the presence of varying amounts of streptomycin and in its absence.

Materials and Methods

The tissue culture procedure about to be described was developed as a means of following in a completely quantitative manner the intracellular survival of an organism. With this procedure it was possible to determine simultaneously both the extracellular and intracellular bacterial concentration as well as the leucocyte population. Streptomycin was employed to prevent extracellular multiplication, thereby ruling out the complication of continual phagocytosis. The use of special tissue culture chambers not only facilitated the use of coverslip cultures, but also permitted an entire test condition to be performed within one vessel, thereby minimizing effects due to variations in experimental conditions.

Tissue Culture Chambers.—These chambers were fabricated from plexiglas with the dimensions as shown in Fig. 1. The base is of ¼ inch stock, 19 mm. wide and 116 mm. long.
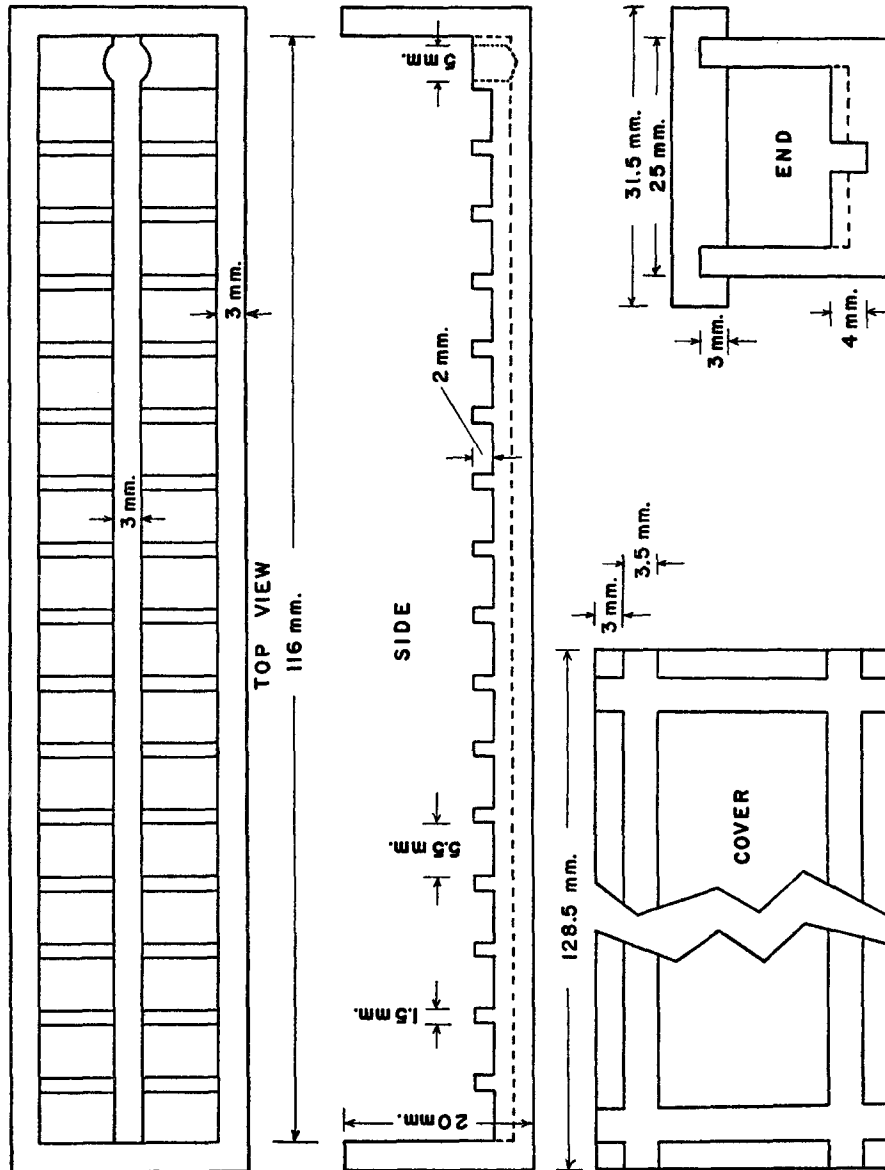

FIG. 1. Plastic tissue culture chamber.

Three mm. from one end and midway between the sides, a 5 mm. hole is drilled to a depth of 4.5 mm. A 3 mm. wide groove is then cut longitudinally along the center of the base (and through the hole) to a depth of 4 mm. This groove serves as a drain and the culture fluid may easily be removed by placing the tip of a 10 ml. pipette into the end hole and aspirating off the fluid.

Transversely across the base 16 other grooves are cut to a depth of 2 mm., leaving 1.5 mm. between adjacent grooves. Each groove is 5.5 mm. wide and will accept a 5 x 18 mm. coverslip. The last groove ends 5.5 mm. from the end of the base where the hole was drilled.

After forming the base the ends are attached by using plexiglas dissolved in chloroform as a cement. The sides are then added in the same manner. The ends and sides are of ⅛ inch stock.

The cover is made of ¼ inch plexiglas, 32 mm. wide and 132 mm. long. Grooves 3.5 mm. wide and 3 mm. deep are cut around the cover to fit and accept the top edge of the chamber The chambers can of course be altered to hold coverslips of any convenient size and number.

The chambers were cleaned by immersing in a solution of haemo-sol[1] and rinsing with ample amounts of tap water followed by distilled water, then drying by draining. The chambers were sterilized by exposing the insides and covers to ultraviolet light for several minutes. Sterile coverslips were aseptically placed in the transverse grooves and the chambers were ready for use.

The chambers may be sealed, if necessary (to prevent loss of CO_2), by applying sterile vaseline or silicone grease to the top edges of the chambers before sealing the covers. In this study, however, the chambers were not sealed unless the cultures were maintained for more than 4 hours without supervision, otherwise the frequent sampling permitted a close check on the pH of the medium.

Staphylococcal Strains.—18-Z: The majority of the experiments were performed with this strain of *Staphylococcus aureus* which was isolated 3 years ago from the human nasopharynx. The organism possesses the following characteristics: bound and soluble coagulase, the alpha and delta hemolysins, hyaluronidase, fibrinolysin, and leucocidin (6). The phage type is 42B/52/80/81. It is sensitive to 0.5 units/ml. penicillin or 1 μg./ml. streptomycin. The strain had been passed serially through 10 rabbits.

P78: This is a strain of *Staphylococcus aureus* which had been maintained as a stock culture since 1932. It possesses the bound and soluble coagulases, the alpha and delta hemolysins, hyaluronidase, and leucocidin, but not fibrinolysin. The organism is not typable with the standard phages. It is sensitive to 1 unit/ml. penicillin and 1 μg./ml. streptomycin. This organism was included in these studies to rule out the possibility that the observed effects were peculiar only to the 18-Z strain.

P77: This is a strain of *Staphylococcus albus* which has also been maintained as a stock culture since 1932. The organism is coagulase-negative.

Tissue Culture Procedure.—Mononuclear exudate cells were induced by the intraperitoneal injection of 30 ml. of mineral oil (Parke, Davis and Co. mineral oil, heavy) into rabbits (or 10 ml. into rats) 2 days prior to the collection of the cells. The peritoneal cavity was washed out with 20 ml. of medium (Hanks solution with 20 per cent homologous serum) containing heparin[2] (1:20,000). Cell counts were made on the original suspensions.

The staphylococcal suspensions were prepared by twice washing with saline a 6 hour old aerated culture in trypticase soy broth. The number of organisms was estimated by reading

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[1] Haemo-sol (Meinecke and Co., Inc., New York) was found convenient since it was rapidly bactericidal for staphylococci, thereby permitting handling of contaminated chambers after a half-hour immersion.

[2] Liquaemin sodium, Organon Inc., Orange, New Jersey.

the saline suspension in a Klett-Summerson colorimeter. Klett readings had previously been equated to actual numbers of staphylococci as determined by chamber counts and plate counts. The washed staphylococci were then mixed with the cell suspension in the ratio of 25 cocci per monocyte. The infected monocyte suspension was then diluted with medium to give a concentration of 1.5 $\times$ 10^6 cells per ml. Six ml. of this suspension were placed into each plastic culture chamber containing coverslips. The chambers were then incubated at 37°C. for 30 minutes in order to permit phagocytosis, settling, and adherence of the cells. After this the fluid was removed from the chambers with a pipette and the chambers washed three times with 6 ml. aliquots of medium. Each chamber then received 6 ml. of medium containing the desired amount of streptomycin; (50 μg./ml. was used as routine unless otherwise stated).

Zero time samples were removed at this time and the chambers then incubated at 37°C. Additional samples were removed at intervals thereafter. The pH of the medium was maintained between 7.0 and 7.4 by the addition of 1.4 per cent sodium bicarbonate solution. Samples were removed and treated as follows. At each sampling time two coverslips and 0.5 ml. medium were removed. The sample of medium was serially diluted with saline and 0.5 ml. aliquots were plated out in trypticase soy agar. At a time when a total of 2 ml. of medium had been removed as samples, the original volume in the chamber was restored by adding more of the same medium. Each coverslip was washed by dipping 3 times in two portions of sterile Hanks solution. One coverslip was fixed in methanol and was later stained with Wright's stain. The other coverslip was placed in a Wassermann tube containing 1 gm. sterile ballotini[3] and 1 ml. sterile 5 per cent saponin[4] solution.

Using a 1 ml. pipette and placing a finger over the upper end to prevent access of fluid, the coverslip was broken so that all pieces were submerged in the ballotini. The entire mixture was then agitated by rotating the tip of the pipette around the bottom of the tube for about 1 minute. Using the same pipette, 0.6 ml. of saponin was drawn up and 0.5 ml. transferred to 4.5 ml. saline. Using separate pipettes, 10-fold dilutions were made in saline and 0.5 ml. aliquots removed for plate counts in trypticase soy agar. Previous studies had indicated that this procedure would result in a quantitative recovery of the intracellular staphylococci. A lytic saponin converts the leucocytes to ghosts and these are then disrupted by the mild grinding action of the ballotini, thus releasing the intracellular staphylococci which are not injured by this procedure. The use of saponin to release intracellular organisms has also been reported by Elberg *et al.* (8).

The coverslip which had been stained with Wright's stain was used to enumerate the total number of cells on the coverslip. This was done by counting the cells in a number of randomly distributed high dry or oil immersion fields equivalent to a total area of approximately 1 square mm. and then calculating the number present on the entire coverslip.

The total number of intracellular staphylococci was determined from the plate counts obtained from the cells disrupted by the saponin and ballotini. The average number of staphylococci per cell was obtained by dividing the total number of intracellular staphylococci by the total number of cells. The number of extracellular staphylococci was followed by the plate counts made on the medium and served as an indicator of the efficacy of the streptomycin in preventing extracellular growth of the organisms.

In some of the earlier studies the intracellular survival was followed by the method of Suter (3). Organism and cell suspensions were prepared as described above and mixed in a ratio of

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[3] Superbrite glass beads, type 100, Minnesota Mining and Manufacturing Co., St. Paul.

[4] Saponin, purified, J. T. Baker Chemical Co., Phillipsburg, New Jersey. Only certain lots will lyse leucocytes; therefore it is imperative that each new lot be tested for activity. Saponins marketed by Merck and Eastman have not been found to be active in this respect.

50 to 100 cocci per cell. Three million infected cells were placed in Porter flasks containing 3 coverslips. The cells were allowed to settle 30 minutes, then were washed, and 1.5 ml. medium added to each flask. At various intervals the coverslips were removed, washed in Hanks solution, fixed in methanol, and stained with Wright's stain. The number of staphylococci per cell was recorded for 100 cells on each coverslip. The distribution of staphylococci within the cell population was then plotted against time.

Cell suspensions consisting primarily of polymorphonuclear leucocytes were obtained by the intraperitoneal injection into rabbits of 30 ml. of sterile Hanks solution and harvesting the cells 3 hours later with 20 ml. of medium containing heparin (1:20,000). Human blood leucocytes were isolated by the method of Skoog and Beck (7) in which heparinized blood was mixed with two volumes of 3 per cent dextran (average m.w. 188,000) in Hanks solution and left to stand 30 minutes in order for settling of erythrocytes to occur. The suspensions were counted, infected, and treated in the same manner as the mononuclear cells.

Direct Microscopic Observations.—Infected cells were placed in small sterile chambers consisting of pyrex rings 18 mm. in diameter and 10 mm. high to which 22 mm. circular coverslips had been attached using sterile silicone grease[5] as an adhesive. A coverslip was attached to one side of the ring, the infected cells were added, allowed to settle onto the coverslip, and then washed with Hanks solution. 0.2 ml. of medium containing streptomycin as required was added and the second coverslip attached. Individual infected cells were continuously observed for periods up to 10 hours with the aid of an inverted microscope equipped with phase contrast optics. The microscope was housed in a plastic cabinet which was automatically maintained at 37°C. Photographs were taken at suitable intervals for permanent records.

EXPERIMENTAL

Intracellular Survival of Staphylococci in Rabbit Monocytes.—Preliminary studies on the intracellular survival of staphylococci made use of the technique developed by Suter.

Infected monocytes were allowed to settle on coverslips in Porter flasks and supplied with medium containing either no streptomycin or 10 to 100 μg./ml. Coverslips were removed at intervals, stained, and the distribution of intracellular staphylococci determined by plotting the frequency of cells containing a certain number of staphylococci. If the organisms were to multiply within the cells then the frequency of cells containing larger numbers of cocci should increase with time, whereas if no multiplication occurred then the relative frequencies should remain the same. If actual destruction of the organisms were to occur, then there should be an increase in the number of cells possessing few or no cocci.

It became evident that if there was no streptomycin to suppress extracellular growth then there was a rapid increase in the number of cells containing large numbers of organisms. Furthermore, there was a rapid destruction of all the leucocytes due to the production of toxins by the extracellular organisms. Fig. 2 illustrates the results obtained under these conditions.

When streptomycin was incorporated into the medium in sufficient concentration to prevent extracellular growth, then there was no change in relative frequencies of intracellular cocci (Fig. 3). These results indicated that if the cellular growth was effectively inhibited by streptomycin there was no increase

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[5] Dow-Corning silicone grease.

in intracellular staphylococci. However, it was not possible to determine by Suter's technique whether the intracellular organisms were living or dead; therefore, one had no way of knowing if the streptomycin could have entered the leucocytes and suppressed the growth of the organisms or if the leucocytes alone could kill, but not necessarily digest, the cocci. It was also possible that the organisms remained viable, but did not multiply. Furthermore, it was sometimes difficult to distinguish and count accurately the intracellular organisms in stained preparations of this type, especially if large numbers of cocci were present in a single cell. These major difficulties were overcome by recover-
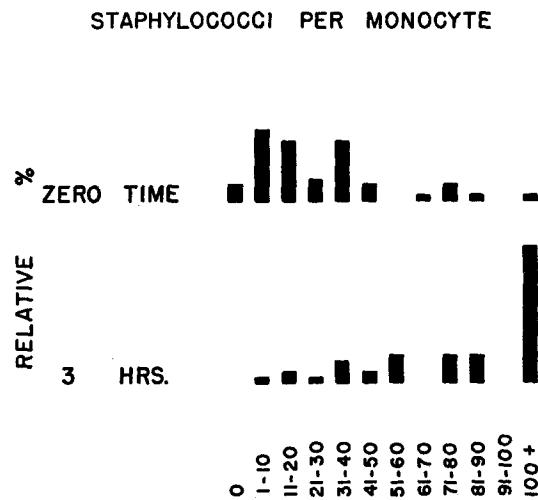
STAPHYLOCOCCI   PER   MONOCYTE

FIG. 2. Distribution of *S. aureus* (18-Z) within rabbit monocytes in the absence of streptomycin in the medium. After 3 hours all leucocytes were destroyed owing to the toxins produced by the organism. The cells were initially mixed with staphylococci in the ratio of 1:50.

ing the intracellular organisms from cells lysed with saponin and ballotini and enumerating them by plate counts.

Infected monocytes were placed in the plastic chambers containing coverslips. The medium contained either no streptomycin or 50 μg./ml. Intracellular and extracellular staphylococci were enumerated by plate counts. The results of such an experiment are shown in Fig. 4.

It can be seen that if no streptomycin was in the medium the organisms grew without difficulty and the average number of intracellular cocci began to increase at the time when the concentration of cocci in the medium had risen to 10^5–10^6/ml. It should be noted that although the upper portion of the curve showing the average number of staphylococci per monocyte would seem to indicate several hundred organisms per cell, this was not really the case. Although intact cells can contain as many as 200 to 300 cocci, many cells at this time were disintegrating, but the cocci remained in the cellular debris and grew

into large collections. In this particular experiment this occurred after about 8 hours.

When streptomycin was incorporated into the medium the average number of viable staphylococci per cell remained essentially constant. Therefore, these results confirmed those obtained with Suter's technique. However, there was still the possibility that somehow the streptomycin might penetrate the leuco-
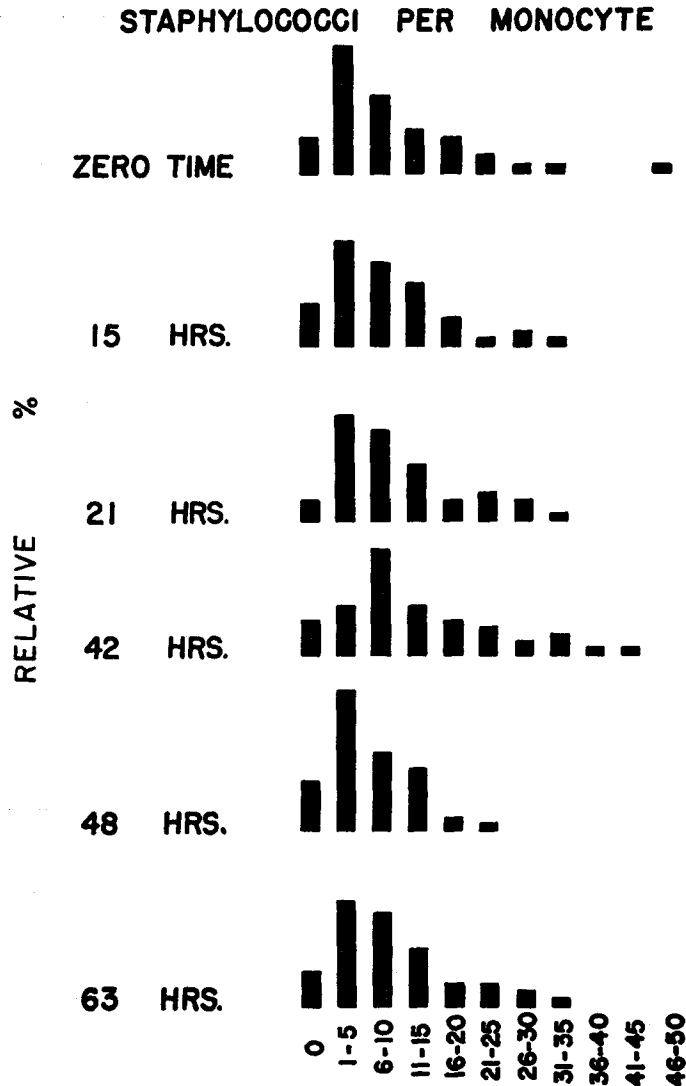

FIG. 3. Distribution of *S. aureus* (18-Z) within rabbit monocytes in the presence of 50 µg. streptomycin/ml. medium. The initial ratio of staphylococci to cells before phagocytosis was 50:1.

cytes in sufficient concentration to prevent multiplication of the cocci. If streptomycin served only to suppress the number of extracellular staphylococci so as to prevent continual phagocytosis, then the same effect should be accomplished without streptomycin by merely washing away the extracellular organisms as rapidly as they multiplied.

Experiments were performed in which infected monocytes were placed in medium without streptomycin. Every hour the medium was removed and the chambers washed twice with
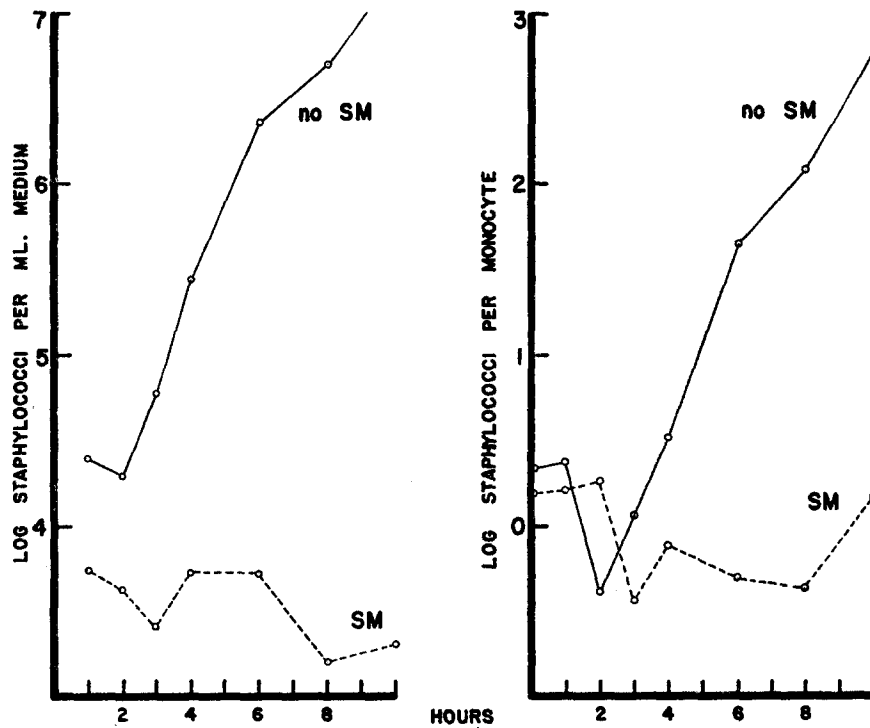

Fig. 4. The intracellular survival of *S. aureus* (18-Z) in rabbit monocytes in the presence or absence of streptomycin (SM).

6 ml. aliquots of fresh medium before the medium was replaced. Samples of medium were taken before and after washing. At 4 and 9 hours all the remaining coverslips were aseptically removed from the chambers, dipped several times in fresh medium, and transferred to new chambers. After 9 hours the washing was discontinued and the organisms allowed to grow at will. Control cultures which were not washed and some which did receive streptomycin in the medium were also included. The results of such an experiment are presented in Fig. 5.

In the control chamber (no streptomycin, no washing), the organisms grew out in the medium in the usual manner and the number of intracellular organisms began to increase after 4 hours when the extracellular concentration of

cocci was about 10^6/ml. In the chamber containing streptomycin the extra-
cellular population decreased steadily and the intracellular organisms remained
essentially constant. In the chamber in which the cells were periodically washed
the number of organisms in the medium was held below the critical level
(10^5 to 10^6/ml.) for about 9 hours; thereafter the staphylococci began to multi-
ply in the usual manner. The intracellular organisms also remained essentially
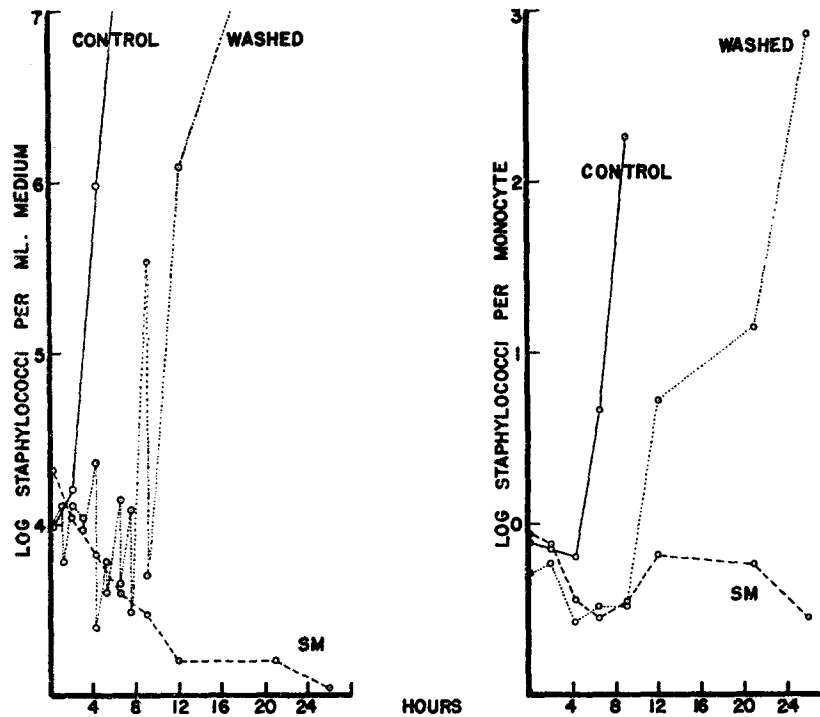

Fig. 5. The intracellular survival of *S. aureus* (18-Z) in rabbit monocytes. Control: no
streptomycin, no washing; washed: no streptomycin, but medium changed periodically; SM:
50 μg streptomycin/ml. medium.

constant for about 9 to 10 hours and then began to increase. Therefore, it was
clearly evident that as long as the number of extracellular organisms was kept
low there was no change in the intracellular population. Washing achieved the
same results as did the use of streptomycin; therefore, the antibiotic obviously
was not solely responsible for the inability of the staphylococci to multiply
within the monocytes.

Similar results were obtained in one of several experiments in which varying
amounts of streptomycin were used in the medium. This experiment is repre-
sented in part by Fig. 6 in which the results of 1 and 100 μg. streptomycin/

ml. are illustrated. When the larger concentration of streptomycin was used the extracellular organisms were suppressed and there was no change in the intracellular cocci; however, when only 1 μg. streptomycin/ml. was used, the extracellular organisms first decreased in number, but eventually streptomycin-resistant mutants[6] grew out in the medium. Again as the number of extra-cellular cocci approached a concentration of 10^5 to 10^6/ml. there was a simultaneous increase in the number of intracellular organisms, but as can be seen from
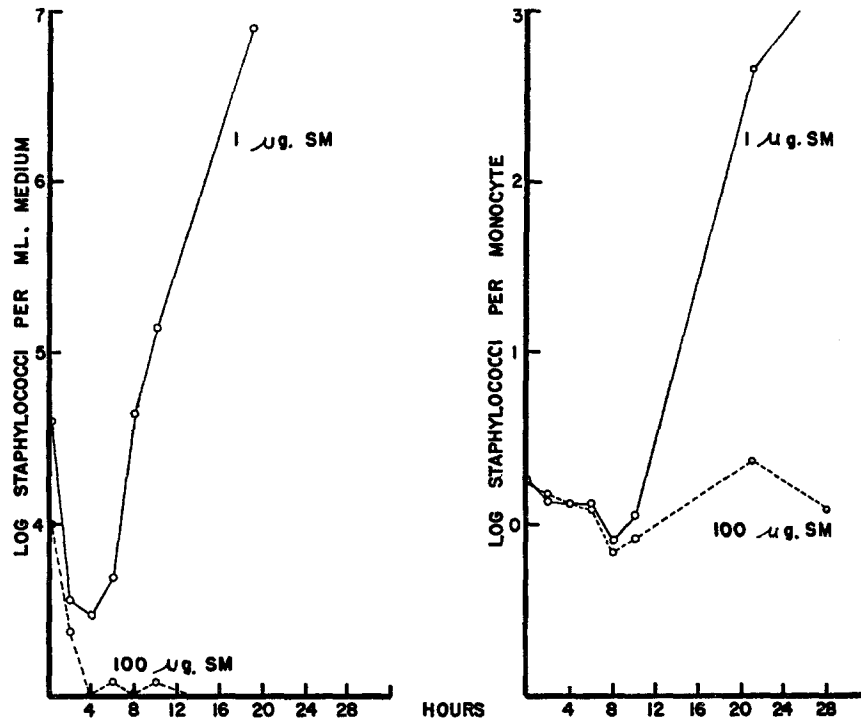

Fig. 6. The intracellular survival of *S. aureus* (18-Z) in rabbit monocytes in the presence of 1 μg. streptomycin (SM) per ml. and 100 μg./ml.

the curve, this began only about 10 hours after the start of the experiment. This was about 6 to 7 hours after one found an increase in intracellular cocci when no streptomycin was used in the medium. Therefore it was again evident that there was no increase in the intracellular parasites unless there was first a multiplication in the medium so that continual phagocytosis could occur.

[6] These were confirmed as follows: 10 colonies were picked at random from dilution plates made at each sampling time and replated on trypticase soy agar containing 5 μg streptomycin/ml. All colonies picked from samples taken after 4 hours were resistant to this concentration of streptomycin.

All the aforementioned studies were carried out with the 18-Z strain. In order to be certain that these results were not limited to this particular strain, the P78 strain of *S. aureus* was also examined within monocytes. In addition a strain of *S. albus* (P77) was observed by this technique. Fig. 7 illustrates the results of such a study when streptomycin was employed in the medium. It will be noted that the P78 strain of *S. aureus* behaved in the same manner as the 18-Z strain whereas the *S. albus* was progressively destroyed with an intracellular half-life of about 2.5 hours.
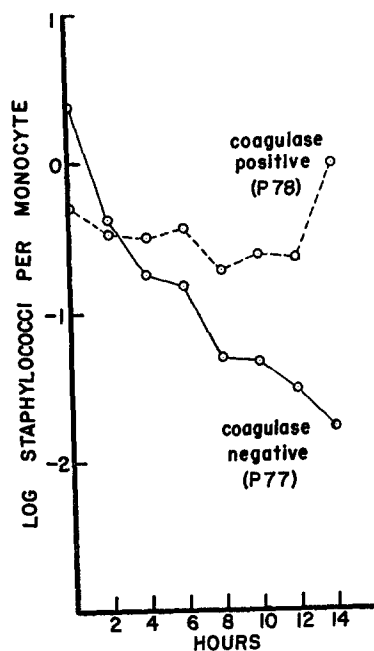

Fig. 7. The intracellular survival of *S. aureus* (P78) and *S. albus* (P77) in rabbit monocytes in the presence of 50 µg. streptomycin/ml. medium.

Intracellular Survival of Staphylococci within Rabbit Neutrophils.—Since it was found that *S. aureus* would survive, but not multiply within rabbit monocytes it was desirable to determine what the organism would do within neutrophils. Rabbit neutrophils were infected with the 18-Z strain and followed in the same manner used for monocytes. Fig. 8 illustrates exactly the same phenomenon, namely, that if the organism were allowed to multiply in the medium, the neutrophils would continue to phagocytize them until the cells were destroyed, whereas, if the extracellular cocci were inhibited by streptomycin, the intracellular population remained constant.

Direct Microscopic Observations.—Both infected rabbit monocytes and neutrophils were studied.

Single cells were continuously observed for periods up to 10 hours with the aid of an inverted microscope equipped with phase contrast optics and enclosed in a plexiglas cabinet maintained at 37°C. In no instance was the 18-Z strain of *S. aureus* seen to multiply within the leucocytes. If no streptomycin was included in the medium the organisms would multiply in the medium, and phagocytosis of the extracellular cocci was observed to proceed until the organisms had multiplied to such an extent that the leucocytes were destroyed as a result of toxins produced. In some cases no streptomycin was used in the medium, but the chambers were washed about twenty times so that the center of the chambers remained free of extra-
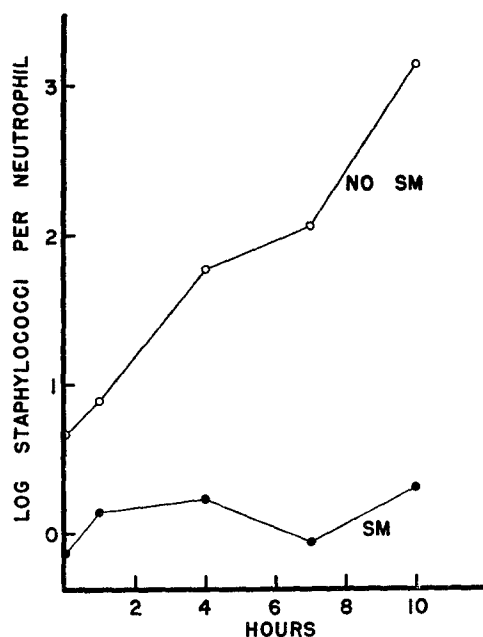

FIG. 8. The intracellular survival of *S. aureus* (18-Z) within rabbit neutrophils in the presence of 50 µg. streptomycin (SM) per ml, medium and in the absence of streptomycin.

cellular staphylococci for periods up to 8 to 10 hours. Under these circumstances phagocytosis was not observed; neither did the intracellular cocci multiply.

When the P77 strain of *S. albus* was observed there was no multiplication of intracellular cocci, but neither were intracellular cocci visibly digested within 10 hours, although according to the previous results the cocci were being killed within the cells.

Although the preparations were photographed at frequent intervals, the photographs often did not reveal all the intracellular cocci within a cell since the organisms would lie in different planes which were beyond the focal capabilities of an oil immersion objective. It is for this reason that photographs have been omitted.
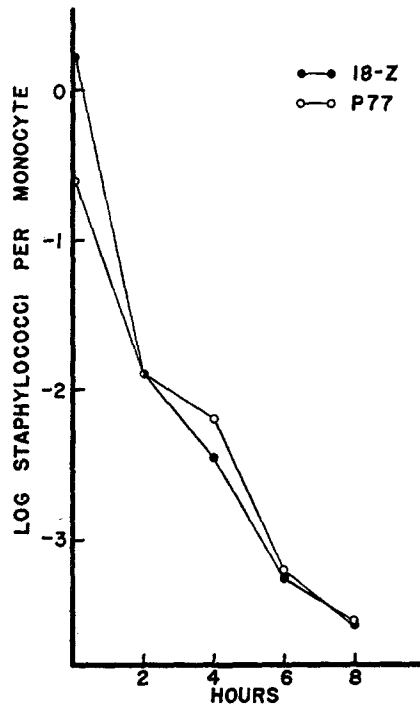
FIG. 9. The intracellular survival of *S. aureus* (18-Z) and *S. albus* (P77) within rat monocytes in the presence of 50 μg. streptomycin/ml. medium.
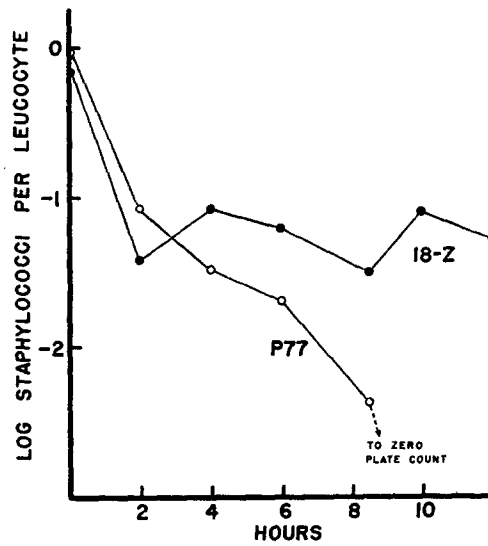

FIG. 10. The intracellular survival of *S. aureus* (18-Z) and *S. albus* (P77) within human blood leucocytes in the presence of 50 μg. streptomycin per ml. medium.

Intracellular Survival within Rat Monocytes.—

Rat monocytes isolated from peritoneal exudates were infected with either *S. aureus* (18-Z strain) or *S. albus* (P77). The results of a typical experiment are shown in Fig. 9. In this case both the *S. aureus* and *S. albus* were progressively destroyed by the monocytes when streptomycin was employed in the medium to prevent extracellular growth of the organisms.

Survival within Human Blood Leucocytes.—

Leucocytes were separated from heparinized human blood by the method described by Skoog and Beck (7). The cell suspensions were infected in the usual manner with either the 18-Z strain of *S. aureus* or the P77 strain of *S. albus*. No attempt was made to separate the various types of leucocytes at this time. The cell suspensions consisted of approximately 80 per cent neutrophils, 15 per cent monocytes, and 5 per cent lymphocytes. The results of a representative experiment are shown in Fig. 10 when streptomycin was used in the medium.

Again it can be seen that the *S. aureus* strain survived within the leucocytes, but did not multiply, whereas *S. albus* was progressively destroyed.

DISCUSSION

These investigations have demonstrated that virulent staphylococci could survive, but not multiply, in leucocytes of normal[7] rabbits and humans. *S. albus* under the same conditions, however, was destroyed by the leucocytes. Rat monocytes, on the other hand, were able to destroy the virulent *S. aureus* as well as the *S. albus*. It has been shown that unless one effectively prevents extracellular multiplication of the organisms, entirely fallacious results are obtained since the effects of continual phagocytosis will be misinterpreted as intracellular multiplication.

The quantitative recovery of intracellular staphylococci has permitted a more critical appraisal of events subsequent to phagocytosis of staphylococci by the leucocytes. Although the fate of staphylococci within rabbit leucocytes has not been adequately reported in the literature, there have been studies dealing with human and rat leucocytes (1, 2). The present authors agree with the findings of Rogers and Tompsett that *S. aureus* can survive within human leucocytes, but disagree with their statement that intracellular multiplication may also occur. It is not apparent from their published results why these workers made this statement that multiplication occurred when all their data seemed to indicate survival only.

The present authors also disagree with Baker's conclusions in which it was claimed that *S. aureus* would survive and multiply inside rat monocytes. The idea that the organisms multiplied was derived from small fluctuations in the

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[7] Sera from leucocyte donors were tested for the presence of circulating precipitating antibodies against the test organisms by the method described by Elek and Levy (9). In no instance were these donor sera found to give flocculation lines; therefore the donors were assumed to represent normal individuals.

extremes of the frequency distribution of staphylococci in stained preparations. However, since there was such a low degree of infectivity in the cell population (about 1 monocyte per 1000 being infected), these distribution patterns were based on 30, or less, infected cells per sample. This would certainly appear to be a rather meager sample from which to draw such a conclusion when it would be expected that even much larger samples would show a considerable variation in the extremes. Furthermore, Baker actually did present data which demonstrated a progressive destruction of intracellular staphylococci when streptomycin was incorporated into the medium, but he chose to interpret this as meaning that the antibiotic rather than the monocytes had destroyed the organisms.

It is possible to speculate that the reason for the high degree of natural resistance of rats toward staphylococci is a consequence of the ability of their leucocytes effectively to destroy the organism after phagocytosis. However, it should be noted that the ability of leucocytes to prevent multiplication even though they cannot destroy the organisms also reflects, under normal circumstances, a rather high degree of resistance. The situation is reminiscent of the state of resistance reported in tuberculosis and brucellosis after the host has been effectively "immunized." Here the organisms are able to multiply in monocytes of normal individuals, but after immunization the monocytes seem able to prevent the pathogens from multiplying intracellularly. This is not to say that conditions might not arise whereby the host's leucocytes could not adequately cope with the organisms and thus permit their unrestricted multiplication. Therefore, it might be well to look for instances in which an individual's "cellular resistance" had been lowered either before or during an infection. Thus it might not be necessary to "increase" an individual's resistance, but merely restore it to its normal state. Studies are currently under way to test this hypothesis.

It might be well to comment on the usefulness of the tissue culture procedure described in this study in elucidating the mechanisms of pathogenesis in staphylococcal disease. Whenever one is fortunate enough to find such a situation in which an organism can survive within leucocytes without multiplying or being destroyed, there is at one's disposal a rather delicate indicator of the intimate relationships between the host cell and parasite. If one selects a strain or organism of known virulence, then one can observe variations in "cellular resistance" by noting whether the organisms can survive, grow, or be destroyed by the cells of the host in question. Conversely if one uses cells from donors of known resistance, one can measure differences in virulence of various strains of the organism. These methods are presently being employed for these purposes in studies dealing with virulence and immunity in staphylococcal infections in humans. There are obvious advantages to by-passing the use of "whole" animals in investigations of this sort.

SUMMARY

A tissue culture procedure is described which permits the quantitative evaluation of the intracellular survival of staphylococci within leucocytes. *Staphylococcus aureus* survived, but did not multiply, within neutrophils and monocytes of normal rabbits. The same was true of normal human blood leucocytes. *Staphylococcus albus* on the other hand was destroyed by these cells under the same conditions. Rat monocytes destroyed *S. aureus* and *S. albus* with equal facility. Although most experiments were carried out in the presence of 50 μg. streptomycin/ml., similar results were obtained without the use of this antibiotic.

The applications of the tissue culture procedure with regard to studies on virulence and immunity in staphylococcal disease are discussed.

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