RELATIONSHIP OF ABSCESS FORMATION IN MICE, GUINEA-PIGS AND RABBITS TO ANTISTAPHYLOCOCCAL ACTIVITY OF THEIR TISSUES AND BLOOD SERUM*

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THE pathogenesis of staphylococcal infections has been difficult to study because of the lack of an experimental model in laboratory animals which mimics the natural human disease. Commonly used techniques include injection of very large doses of cocci by intravenous, intraperitoneal or intracerebral routes. The diseases produced by these methods bear little relationship to the commonest type of human staphylococcal lesion, the localized cutaneous abscess.

Subcutaneous injection of suspensions of staphylococci is likewise open to criticism, since large numbers of cocci are required to initiate infection. Elek (1956, 1957), for example, has shown that from $1–6 \times 10^6$ staphylococci must be introduced subcutaneously or intradermally in order to produce a cutaneous abscess in man. Similar inocula are required in laboratory animals (Panton and Valentine, 1929; Gorrill, 1951). Although these experiments have been taken as evidence that man and laboratory animals are resistant to staphylococcal infection, it seems probable that natural infection of man results not from exposure to such large inocula but from much smaller inocula introduced under favourable conditions; that is, under circumstances affecting local tissue resistance.

Recently, James and MacLeod (1961) described an experimental infection in mice based on Elek's observation (1956, 1957) that the number of staphylococci required to produce an abscess in man is reduced from $10^6–10^2$ when the bacteria are introduced subcutaneously on silk sutures. They were able to produce cutaneous abscesses consistently in mice with as few as 10–100 coagulase positive staphylococci (James and MacLeod, 1961).

The present studies were undertaken to evaluate further this experimental model by applying it to other species of laboratory animals. In addition, susceptibility to infection of various species was compared to the antistaphylococcal activity of their tissues and of their blood serum. Susceptibility shows a remarkable correlation with the bactericidal activity of blood serum and of subcutaneous tissues toward *Staphylococcus pyogenes*.

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MATERIALS AND METHODS

Animals.—CFI female mice, 15–20 g. in weight ; albino guinea-pigs of either sex, 500–600 g. in weight ; and male albino rabbits of approximately 2–3 kg. were used.

Sutures.—Standard 4–0 black braided surgical silk cut in 5 cm. lengths was used. Darning and cashmere wool thread of the same length were also used in a few experiments in rabbits.

Cultures.—In most experiments strain 5848, a coagulase positive strain of *Staph. pyogenes* was used. It was isolated originally from a paronychia. Strain 5848 ferments mannitol, forms yellow pigment, produces alpha and delta haemolysins and is phage type 80, 81. A coagulase negative strain (Casiz) of staphylococcus isolated from a contaminated blood culture was also frequently used. Strain Casiz is non-haemolytic on fresh rabbit blood agar plates, produces a white pigment and ferments mannitol. Other strains tested included : Smith (coagulase positive, non-typable), 8089 (coagulase positive, phage type 52A, 80, 81), B.Z. (coagulase positive, phage type 80, 81), Bargan (coagulase positive, phage type 80, 81), De Leo (coagulase positive, phage type 29, 52, 52A, 79, 80) and Holstein (coagulase positive, non-typable). All strains were maintained on trypticase soy agar slants in a refrigerator at 4°–8°.

Culture media.—Trypticase soy agar and broth were used in all experiments.

Preparation of animals.—On the day prior to inoculation, the backs of guinea-pigs and rabbits were shaved, and a commercial depilatory lotion (Neet®) was applied to remove the remaining hair. Mice were anaesthetized with 0·1 to 0·15 ml. of 0·7 per cent veterinary Nembutal® injected intraperitoneally. A depilatory (Nair®) was applied directly without shaving.

Method of infecting animals.—The method is essentially that described by James and MacLeod (1961). Sutures were placed in 10 ml. of an appropriate broth dilution of an 18-hr. broth culture and incubated for 30 min. at 37°. They were then removed and dried on sterile filter paper at room temperature.

The total number of staphylococci that could be cultured from a suture was about 0·1 per cent of the total number present in 1 ml. of the broth dilution. Thus, if the concentration of bacteria was 10^9 per ml. of broth, the sutures would pick up about 10^6 cocci.

Sutures were inserted subcutaneously on the back with curved surgical needles. In mice, about 1–1·5 cm. of suture was inserted subcutaneously, while in guinea-pigs and rabbits, 3·5–4 cm. was inserted. The ends of the suture just above the skin were capped with collodion. In mice, the ends were cut and samples saved for calculation of the dose ; in guinea-pigs and rabbits, most of the suture was left beneath the skin, and the ends were not cut off. For these experiments in which bacterial counts were to be made on the sutures subsequent to their removal from the animal, the entire suture was inserted into the subcutaneous tissue. A single suture only was put into a mouse ; 4–6 sutures were placed in guinea-pigs ; and 8–12 in rabbits. In the latter species, sutures were placed at a minimum distance of 1 in. apart.

Estimation of subcutaneous dose.—Two unused sutures from each culture dilution were placed in 1·5 ml. of broth and were agitated on a reciprocating shaker for 45 min. at room temperature. Serial dilutions of the broth were made, and 0·1 ml. aliquots were dropped on the surface of agar plates, spread, allowed to dry and incubated at 37° overnight. Plates with between 20 and 200 colonies were counted.

When the sutures were inserted in mice, sample ends were cut off and counted as well. The counts on whole sutures minus the counts on the ends were taken as the subcutaneous dose. In guinea-pigs and rabbits nearly the entire suture was left subcutaneously, hence the ends were not counted.

In some instances, sutures were removed at intervals after insertion and the number of live staphylococci counted after shaking the suture in 1·5 ml. of sterile distilled water for 45 min. as described above. Distilled water was used as diluent in order to lyse the leucocytes and release intracellular cocci.

In other experiments, sutures were removed, and the exudate coating them was smeared on glass slides and stained with MacNeal's tetrachrome stain.

Bactericidal activity of serum.—Whole blood was allowed to clot at room temperature. The clot was rimmed, permitted to retract at 4° and the serum removed following centrifugation. To 2·0 ml. of serum was added 0·02 ml. of a 10^{-3} dilution in broth of an 18-hr. broth culture of the staphylococcal strain and the mixture incubated at 37° in a water bath. Samples were removed hourly at the first through the 5th hr. and at 24 hr. Dilutions were

spread on agar plates and incubated for approximately 18 hr. at 37°. The colonies of staphylococci were then counted.

The results of bactericidal tests were the same whether serum was used fresh or after heating at 56° for 30 min. Lack of dependence on complement, as well as various other properties, indicates that the active material can be identified with the so-called heat stable bactericidins of serum.

Bactericidal activity of whole blood and plasma.—Whole blood and plasma were prepared using heparin as anticoagulant. To 10 ml. of blood was added 1 ml. of heparin containing 1000 units. In a few experiments sodium citrate in a final concentration of 0·4 per cent was used in place of heparin. The bactericidal power was tested in a manner similar to that used for serum.

RESULTS

Infections in guinea-pigs

Guinea-pigs were readily infected by means of sutures contaminated by *Staph. pyogenes*. Table I illustrates the infection rate for a group of animals at various dosages by strain 5848 (coagulase positive, phage type 80, 81). Cutaneous abscesses were produced consistently in most animals with as few as 10–100 staphylococci. In the absence of a suture, the dose required to produce subcutaneous abscesses in the guinea-pig is approximately 10^7 cocci.

TABLE.—*Response of Guinea-pigs and Rabbits to Subcutaneously Introduced Silk Sutures Carrying Various Strains of Coagulase Positive Staphylococci*

Strain	Number of staphylococci introduced	Rabbits		Guinea-pigs	
		Number tested	Number positive	Number tested	Number positive
5848	10^1	4	0	9	6
	10^2	6	0	14	10
	10^3	6	0	15	13
	10^4	9	0	11	9
	10^5	8	0	11	8
	10^6	8	0	..	..
Smith	10^1	..	..	2	0
	10^2	..	..	2	2
	10^3	..	..	2	2
	10^6	6	0	..	..
8089	10^1	..	..	3	1
	10^2	..	..	3	3
	10^3	..	..	3	3
	10^6	6	0	..	..
BZ	10^6	6	0	..	..
Bargan	10^6	5	0	..	..
DeLeo	10^6	5	0	..	..
Holstein	10^6	4	0	..	..

Positive = Abscess formation
0 = No abscess formation
.. = Not tested

Within 8 hr. after the contaminated suture was introduced, erythema was visible at the points where it pierced the skin. Sutures removed at this time were coated with a shiny, cream coloured exudate which when smeared on glass slides revealed numerous intact polymorphonuclear leucocytes (PMN) with

visible intracellular cocci. Histologically, there was a polymorphonuclear leucocyte infiltrate surrounding the suture, as well as oedema and a diffuse PMN infiltrate in the adjacent subcutaneous tissue.

At 16 hr. erythema was often visible along the entire course of the suture. Smears of the exudate on the sutures showed that many of the leucocytes were partially or completely disrupted and that there were visible extracellular staphylococci, as well as cocci within the few remaining intact cells. Sections of the lesions showed a more extensive infiltrate about the suture with fibrinous encapsulation. Some necrosis of PMN was present. In the surrounding subcutaneous tissue, the infiltrate was more extensive than at 8 hr. and involved the adjacent muscle layer.

At 24 hr. swelling was usually present along the course of the suture. Cells in the exudate smeared from the suture were completely disrupted, and most of the staphylococci were extracellular.

At 48 hr. there was considerable swelling although the erythema had faded in most animals. Pus could often be expressed by compressing the lesion. A typical furuncle was formed at the site of the entrance and egress of the suture in an occasional animal. Histologically, necrosis of PMNs was evident with a fibrinous wall forming about the suture and its infiltrating exudate. The gross lesion then progressed and often drained spontaneously by the 4th or 5th day. Histologically, the abscess gradually became walled off, and the PMN infiltrate was replaced by chronic inflammatory cells.

The histological reaction of sterile silk resembled that to the contaminated sutures for the first 48 hr., except for earlier walling off and infiltration by macrophages and lymphocytes. There was no gross evidence of inflammation.

Guinea-pigs were also infected by the suture technique with the following coagulase positive strains : Smith (non-typable) and 8089 (52A, 80, 81). Lesions produced by these strains were indistinguishable from those described above. There was no evidence of a significant difference in virulence among the three strains with the limited testing that was employed.

Sutures contaminated with coagulase negative staphylococci failed to produce abscesses although an occasional guinea-pig exhibited small amounts of exudate along the course of the suture.

Failure to induce abscesses in rabbits

Despite the ability of strain 5848 to produce localized cutaneous abscesses in rabbits when 10^7 cocci suspended in broth were injected subcutaneously, no grossly discernible lesion was observed when this strain was introduced on sutures at doses ranging from 10^2–10^6 cocci, the latter being the maximum number which can adhere to a suture. Transient erythema was often observed along the course of the suture, but this never progressed to the presence of swelling or to abscess formation. Sutures removed at 8 and 16 hr. after insertion were covered with a white tenacious exudate which consisted of amorphous eosinophilic material with only an occasional intact PMN visible. Histologically, there was infiltration of PMN about and within the suture which was well encapsulated by 24 hr. The reaction about the suture persisted at 48 hr., but the inflammatory response in the nearby subcutaneous tissues, which had been prominent at 24 hr., had diminished markedly with replacement of the PMN by macrophages and lymphocytes. Frequently by 48 or 72 hr. the suture had

been extruded from the subcutaneous tissue with no grossly visible evidence that it had ever been there. The histological response to sterile silk sutures was similar to that seen with staphylococcus-coated sutures up to 24 hr. after introduction. Subsequently, resolution occurred more rapidly than when staphylococci were present.

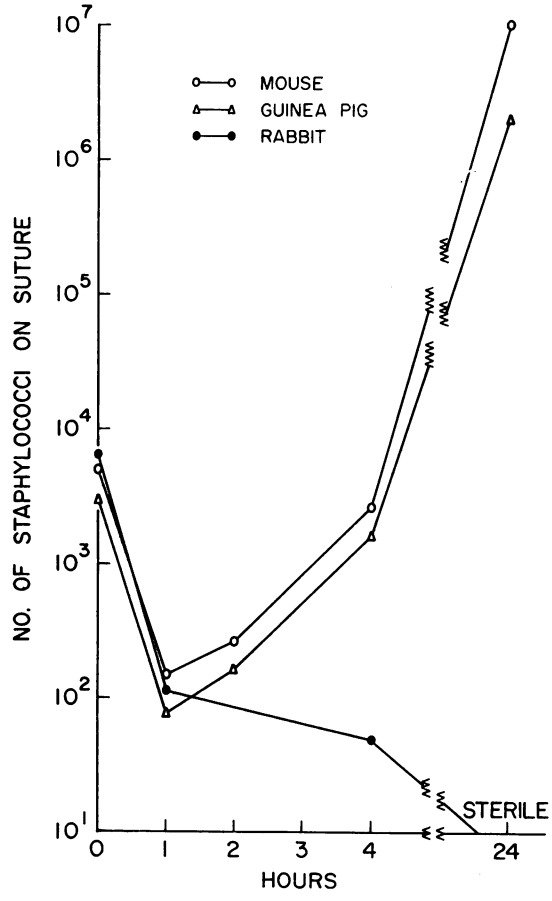

FIG. 1.—Survival *in vivo* of coagulase positive staphylococci on silk sutures introduced subcutaneously in mice, guinea-pigs and rabbits.

As in the case of strain 5848, other coagulase positive strains of staphylococci failed to produce significant lesions in rabbits (Table I). Sutures contaminated with coagulase negative staphylococci also failed to produce abscesses in rabbits.

No infections were observed when contaminated wool sutures were substituted for silk sutures (Colbeck, 1960).

Fate of staphylococci on sutures introduced in mice, guinea-pigs and rabbits

Because of the unexpected resistance of rabbits to infection by the silk suture technique, an attempt was made to compare the survival and growth of coagulase

positive staphylococci on sutures in mice, guinea-pigs and rabbits. Sutures were removed from the subcutaneous tissues at intervals, placed in a sterile distilled water, shaken for 45 min. to lyse the cells and release phagocytosed cocci, and then counted as described above. The results are shown in Fig. 1.

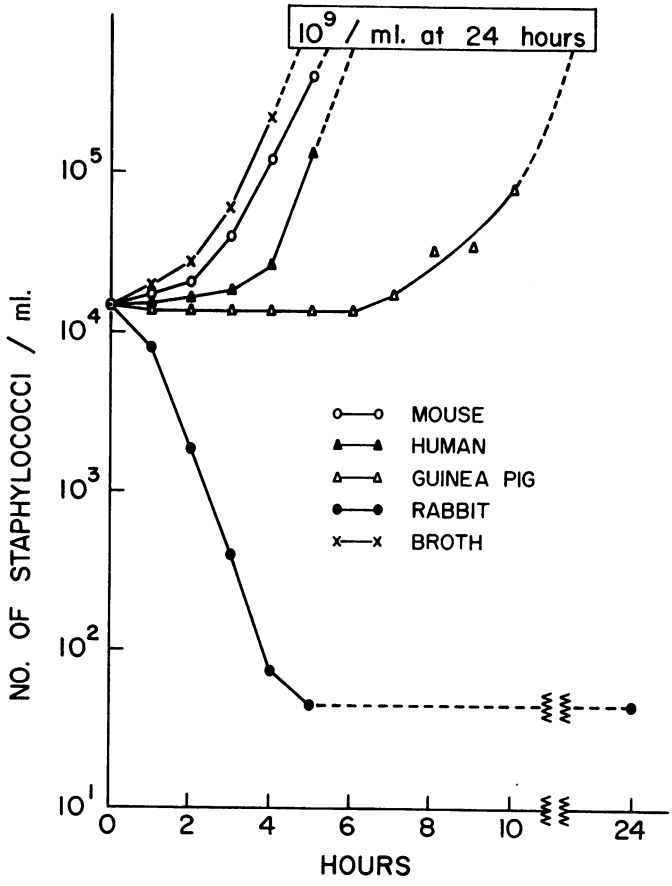

FIG. 2.—Bactericidal activity of blood serum of mouse, guinea-pig, rabbit and man for coagulase positive staphylococci. In the case of serum of mouse, guinea-pig and man, the bactericial counts had reached 10^9 per ml. at 24 hr.

Mice and guinea-pigs responded similarly with an initial decline in the number of culturable staphylococci during the first hour of from 10–100-fold (the initial count ranging from 10^3–10^5 cocci). Subsequently there was a rapid increase in the suture counts so that at 24 hr. approximately 10^7 organisms could be cultured from each suture. In striking contrast to the results in mice and guinea-pigs, sutures placed in rabbits showed a rapid decline in the number of viable staphylococci, there being less than 10^2 bacteria per suture at 4 hr. and only an occasional staphylococcus remained alive at 24 hr.

Serum bactericidal activity

Experiments with whole blood and plasma gave results which could not be interpreted because in the concentrations required to prevent clotting, citrate inhibited the growth of staphylococci and heparin inhibited the bactericidal activity of serum.

The bactericidal power of suspensions of leucocytes of the four species was not measured in the present study because it was felt that the amount of washing needed to free the leucocytes from serum factors would lead to changes in the properties of the cells, especially those of the polymorphonuclear series, which would make the results difficult to analyse. Earlier studies by Cohn and Morse (1959) and by Melly, Thomson and Rogers (1960) have shown that leucocytes from rabbit and man have bactericidal activity for *Staph. pyogenes* although killing is never complete.

Serum antistaphylococcal activity varied considerably among the species tested (Fig. 2). Mouse serum supported the growth of staphylococci in a manner indistinguishable from that of nutrient broth. By the end of 5 hr., the count had increased from 10^4–10^7 per ml. and growth continued exponentially until the count reached approximately 10^9 cells per ml. Guinea-pig serum was bacteriostatic for a 5-hr. period, while human serum maintained bacteriostatic activity for 3–4 hr. Following this, rapid growth occurred in the serum of guinea-pig and man, and by 24 hr. the count had reached 10^9 per ml.

Rabbit serum, on the other hand, showed consistent and marked bactericidal activity. Decrease in the number of staphylococci by a factor of at least 100 was observed consistently within the first 3 hr. The activity persisted for 24 hr. by which time the number of staphylococci was almost always less than 10^2 per ml.

In most of the tests of serum bactericidal activity of the four species, a single strain of *Staph. pyogenes* (5848) was used. Qualitatively similar results were obtained with several other strains of *Staph. pyogenes*. When a coagulase negative strain was used in the bactericidal tests, the activity of the serum of the four animal species was similar to that observed with coagulase positive strains.

DISCUSSION

In order to produce subcutaneous abscesses in guinea-pigs in the absence of a silk suture, approximately 10^7 staphylococci must be injected. This dose is of the same order of magnitude as that required to cause abscesses when injected subcutaneously into " suture susceptible " man (Elek, 1956 ; Elek and Conen, 1957) and mouse (James and MacLeod, 1961). It is of particular interest that a similar dose of staphylococci (10^7) causes abscesses when injected as a suspension subcutaneously in rabbits although this species is completely resistant to abscess formation when as many as 10^6 staphylococci are introduced on a suture. In other words, all four species behave in the same way in that abscesses form when similar large doses of staphylococci (*ca.* 10^7) in suspension are injected subcutaneously. However, when the cocci are introduced on silk sutures, the rabbit is resistant to the largest dose that can be obtained by this technique (10^6), whereas in the other three species, abscesses form at suture doses of about 10–100 cocci.

James and MacLeod (1961) observed that the ability of different suture

materials to potentiate infections in mice appeared to be related to the inflammatory response evoked by the suture alone. The greater the inflammatory response to the suture material, the fewer accompanying staphylococci were needed to produce an abscess. The effect of the suture and the associated inflammatory response may be to protect the introduced staphylococci from the host's defences which allows multiplication to numbers large enough to produce a lesion independent of the suture. Assuming uninhibited growth (with a generation time of about 30 min.), an inoculum of 10^2 cocci increases to 10^6 in less than 7 hr.

In both the guinea-pig and the rabbit, the histological reaction to the sterile suture was similar to that of the staphylococcal-coated suture during the first day. However, the extent of the initial inflammatory response does not by itself account for the striking species variation. Although the inflammatory response in the rabbit was less marked than in the guinea-pig, it was still significant.

Tests of survival of staphylococci introduced on sutures revealed a remarkable difference in the tissue antistaphylococcal activity of the rabbit as compared to the mouse and guinea-pig. In the latter 2 species, following a lag period of 2–3 hr., rapid growth occurred, whereas in the resistant rabbit, the total number of staphylococci on the suture fell rapidly so that sutures remaining in the animal for 24 hr. were almost invariably sterile.

A striking parallelism exists between the survival and growth of staphylococci in the tissues, the antistaphylococcal activity of serum and the susceptibility to suture-induced infection of the various species. Staphylococci increased rapidly in numbers when inoculated in mouse serum and only slightly less rapidly in guinea-pig and human sera. Similarly, growth in the tissues of mice and guinea-pigs in association with silk sutures was rapid. In the rabbit, the rapid killing of staphylococci introduced on sutures and the failure of abscess formation to occur are paralleled by the marked bactericidal activity of rabbit serum.

Because of the risk involved (Elek, 1956), studies on tissue antistaphylococcal activity of man have not been carried out in order to determine whether it parallels the low level of serum antistaphylococcal activity demonstrated in the present study and the great susceptibility of man to abscess formation when small numbers of staphylococci are introduced on silk sutures (Elek, 1956; Elek and Conen, 1957).

The association of resistance to staphylococcal infection and antistaphylococcal activity of blood is not limited to rabbits. Rammelkamp and Lebovitz (1956) reported that injection of staphylococci into chickens, ducks and geese does not result in abscess formation and that all three species possess antistaphylococcal activity in their blood in the absence of cellular elements. However, their report contains insufficient information about the strain used and the method of measuring antistaphylococcal activity, so that it is not possible to decide whether the behaviour in these species is comparable to that in the rabbit.

Colbeck (1960) reported that rabbits develop abscesses when wool sutures contaminated by staphylococci are introduced in the skin and subcutaneous tissues. Attempts in this laboratory to confirm Colbeck's results, employing two different types of wool, were unsuccessful.

SUMMARY

Superficial abscesses are produced in the guinea-pig by the introduction of small numbers (10–100) of coagulase positive staphylococci on silk sutures. This is similar to the suture dose previously found effective in mouse and man.

Rabbits are uniformly resistant to infection at the largest dose that can be taken up by a suture (10^6 cocci).

In man, rabbit, guinea-pig and mouse approximately the same dose of *Staph. pyogenes* (10^6) causes subcutaneous abscesses when injected as a suspension. This method, therefore, does not reveal the marked differences in species susceptibility that become apparent when the cocci are introduced on silk sutures. Man, guinea-pig and mouse are highly susceptible, whereas rabbit is resistant.

The bactericidal activity of blood serum of mouse, guinea-pig and rabbit correlates with the ability of staphylococci to survive and multiply when introduced into the subcutaneous tissues of these species on a suture and also with the ability of staphylococci to cause abscess formation when introduced in this way.

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