INDUCTION OF STAPHYLOCOCCAL INFECTIONS IN MICE WITH SMALL INOCULA INTRODUCED ON SUTURES*

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Received for publication January 3, 1961

THESE studies were undertaken in the hope of evolving a simple experimental model for staphylococcal infection, the aim being to employ a small inoculum and to approximate natural infection of man in type and site of lesion.

Most of the present methods of producing experimental staphylococcal infection involve techniques which are probably not analogous to the pathogenesis of natural infection. The routes used, such as intraperitoneal, intravenous or intramuscular injection are highly artificial for a micro-organism which, in man, typically causes localized, superficial pus formation. Secondly, the doses needed to infect by these routes are very high. Of current investigators, Gorrill (1951, 1958), has used the smallest doses, but even in this method intravenous injection of 10^5–10^6 staphylococci is required to induce the formation of kidney abscesses in mice. These inocula are probably much larger than those responsible for the common infections of man.

A consideration that appeared important to us is the frequent association of staphylococcal infection with either a " foreign body " or local damage of some sort. For example, in acne vulgaris, sebaceous secretions may become inspissated, plug the ducts and often rupture them, with extrusion of sebaceous material into the surrounding tissues. Wound infection often begins about sutures with the formation of stitch abscesses. It seems likely that the staphylococcus is not usually a primary invader and that the provision of favourable local circumstances should be taken into account in the design of an experimental model of infection.

Elek and Conen's experiments (1957) on human volunteers suggested a possible line of approach. They found that the minimum pus-forming dose (PFD) of *Staphylococcus pyogenes* in human skin was reduced from 10^6 to about 10^2 when the cocci were introduced on silk sutures. The method described in the present article is essentially that of Elek except that mice were used instead of human volunteers and various other suture materials were used in addition to silk.

MATERIALS AND METHODS

Mice.—CFW female mice of 15–20 g. weight were used one week after arrival.

Suture materials.—Black braided silk, 4–0 gauge, Ethicon Inc.; white cotton thread, size 40 machine twist, J. and P. Coats; black braided nylon, 4–0 gauge, Ethicon Inc.; black braided silicone silk, 4–0 gauge, American Cyanamid Co.; black human hair; green braided

* These studies have been supported in part by a grant from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

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Dacron polyester fibre (Mersilene), 3–0 gauge, Ethicon Inc.; catgut, 4–0 gauge, Ethicon Inc.; steel wire, 4–0 gauge, Ethicon Inc.

All the above materials were boiled for 2–3 min. before use.

Cultures of Staphylococcus pyogenes.—One strain of *Staph. pyogenes* (phage type 52/52A/80/81) was isolated from an infected wound in an experimental dog in a veterinary institution where this strain was prevalent among the personnel. The following cultures were obtained from human sources: 8 strains of phage type 80/81 or 52/80/81; 1 strain of phage type 47/53/54/75/77/VA_4; 1 strain of phage type 3A/3B/3C/55/71; 7 nontypable strains; 4 nontypable stock cultures, probably originally from human sources.

Cultures of coagulase negative Staphylococci.—Eighteen strains isolated from various human sources; 1 strain from an air settling plate in a hospital operating room; 1 stock strain of *Staphylococcus albus* from the Microbiology Department, University of Pennsylvania School of Medicine.

Culture media.—Trypticase Soy Broth was obtained from Baltimore Biological Laboratories. Trypticase Soy Agar was made by incorporating 1·5 per cent agar into the broth. These media were used for phage typing and for propagation of phages. They were employed also for the culture of staphylococci used in the present experiments.

Anaesthetic.—Nembutal, 0·1 ml. of 0·7 per cent solution per 10 g. bodyweight was injected intraperitoneally.

Depilatory.—A commercial cosmetic depilatory (Nair) was used.

Method of inducing infection.—Mice were prepared by depilating under nembutal anaesthesia an area approximately 1·5 cm. square over the shoulder blades.

Sutures were prepared by soaking 2-in. lengths for 30 min. at 37° in an appropriate dilution in broth of an overnight broth culture of a staphylococcal strain. The number of staphylococci picked up by a 2-in. silk suture was about 1/1000 of the number of staphylococci per ml. of suspension; the number picked up by a nylon suture was less, usually about 1/10,000 the number per ml.; for example, a silk suture would pick up 10^4 organisms from a suspension containing 10^7 staphylococci per ml.; a nylon suture would pick up 10^3 staphylococci from the same suspension.

After soaking, the sutures were transferred to sterile filter paper in petri dishes and left at 4° overnight.

The mice were again anaesthetized on the following day and sutures inserted subcutaneously with a fine curved cutting needle, taking a bite of 1·0–1·5 cm. Ends of the suture were cut off just clear of the skin and capped with collodion. The sutures were not tied.

Enumeration of staphylococci on sutures.—The number of staphylococci introduced on the sutures was estimated in the following way. Two unused sutures were put into separate tubes of broth and shaken mechanically for 45 min. at room temperature. Two sets of cut-off ends from the sutures put into mice were treated in the same way and viable counts made on the contents of each tube. The difference between the mean count for the entire suture and mean count for the cut-off ends was taken as the dose inserted.

Bacterial counts were very reproducible and appeared to give good assessment of the dose used provided the numbers to be counted were not too small. The method was tested by shaking sutures repeatedly in fresh tubes of broth and finally cultivating the sutures themselves by stretching them out on agar plates.

Table I gives the results of a typical experiment in which counts were made of staphylococci on sutures. The numbers 1, 2, 3, 4 represent 4 sutures each of which was shaken 3 times. The "working count", derived from the results of the first shaking, is the count given in most of the following experiments in which the sutures were shaken only once. The total count for each suture is calculated from the total number of organisms recovered from 3 successive shakings. All sutures were laid on agar after the 3rd shaking and yielded only a few scattered colonies on incubation. In other words, 3 successive shakings washed most of the staphylococci from the sutures and permitted reasonably accurate enumeration. The results also show that the working count was a fairly good estimate of the dose used.

RESULTS

Silk sutures

The development of the gross lesions is shown in Figs. 1–4. The first visible sign of infection was thickening of the tissues over the suture so that it was no

TABLE I.—*Recovery of Staphylococci from Silk Sutures upon Repeated Shaking in Broth*

	Number of staphylococci per 0·05 ml. broth* Suture			
	1	2	3	4
First shaking	114	106	159	143
Second shaking	65	67	37	35
Third shaking	26	40	28	28
Total number of colonies . .	205	213	224	207
Total count per suture . . .	$6·2 \times 10^3$	$6·4 \times 10^3$	$6·7 \times 10^3$	$6·2 \times 10^3$
Working count (from 1st shaking) .	$3·4 \times 10^3$	$3·2 \times 10^3$	$4·8 \times 10^3$	$4·3 \times 10^3$

Culture : *Staph. pyogenes*, phage type 52/80/81. Sutures were soaked in a suspension containing approximately 10^6 organisms per ml.
Suture : 4–0 silk.

* Sutures were shaken in 1·5 ml. vols. of broth, of which 0·05 ml. was used for viable counts.

longer visible through the skin. This change was apparent 24–48 hr. after insertion of the suture. Infection progressed fairly rapidly and by the 3rd or 4th day there was usually an obvious abscess. Most of these abscesses began to subside by the 5th or 6th day, particularly if they were draining freely. Abscesses which did not drain early increased slowly in size for as long as 2–3 weeks before subsiding. Recovery was always complete by 5 weeks.

The mice were usually killed on the 5th or 6th day. The local lesion was cultured and pus was looked for along the suture. Pus was sometimes hard to find even with quite a large swelling and might be plentiful when the superficial swelling was slight.

Several groups of mice were used for a rough estimate of the dissemination of staphylococci in the body in this type of infection. Spleen and kidneys were removed aseptically and cut into small pieces with a sterile scalpel. Both kidneys from an animal were minced together. A loopful of each type of tissue suspension was then spread on segments of a trypticase soy agar plate and about 0·05 ml. of heart blood was streaked on another segment of the same plate. All cultures of staphylococci recovered from these plates were phage typed.

In this way 370 mice in all were examined between the 5th and 8th day after infection.

EXPLANATION OF PLATES

FIG. 1.—Appearance immediately after insertion of silk suture. Suture is visible through the skin along its entire length. If no infection takes place it remains visible as long as retained.

FIG. 2.—Infection at 2 days. Thickening of skin obscures the suture. This change is sometimes apparent 24 hr. after insertion of the suture.

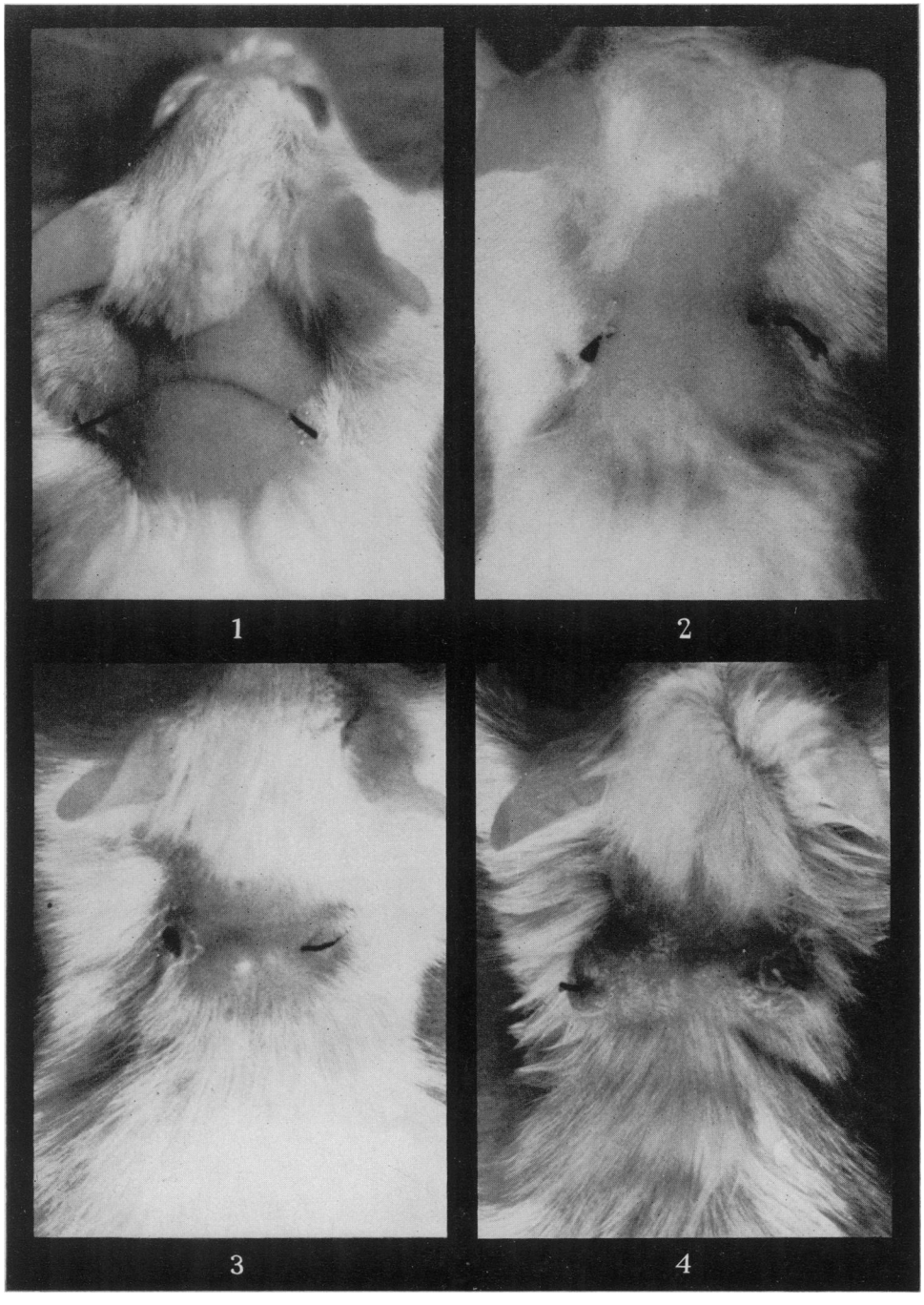
FIG. 3 and 4.—Infection at 4 days. There is usually obvious pus formation.

FIG. 5.—Silk suture carrying *Staph. pyogenes* ; 24 hr. after insertion. There is a marked polymorphonuclear and fibrin exudate immediately surrounding the suture with oedema of skin and subcutaneous tissues. ×35.

FIG. 6.—Silk suture carrying *Staph. pyogenes* ; 3 days after insertion. Necrosis has occurred with extensive oedema and infiltration of surrounding tissues. ×35.

FIG. 7.—Silk suture carrying *Staph. pyogenes* ; 5 days after insertion. Polymorphonuclear infiltration and oedema are extensive in underlying muscle. ×35.

FIG. 8.—Silk suture carrying *Staph. pyogenes* ; 5 days after insertion. Polymorphonuclear infiltration of muscle is shown at higher magnification. ×103.



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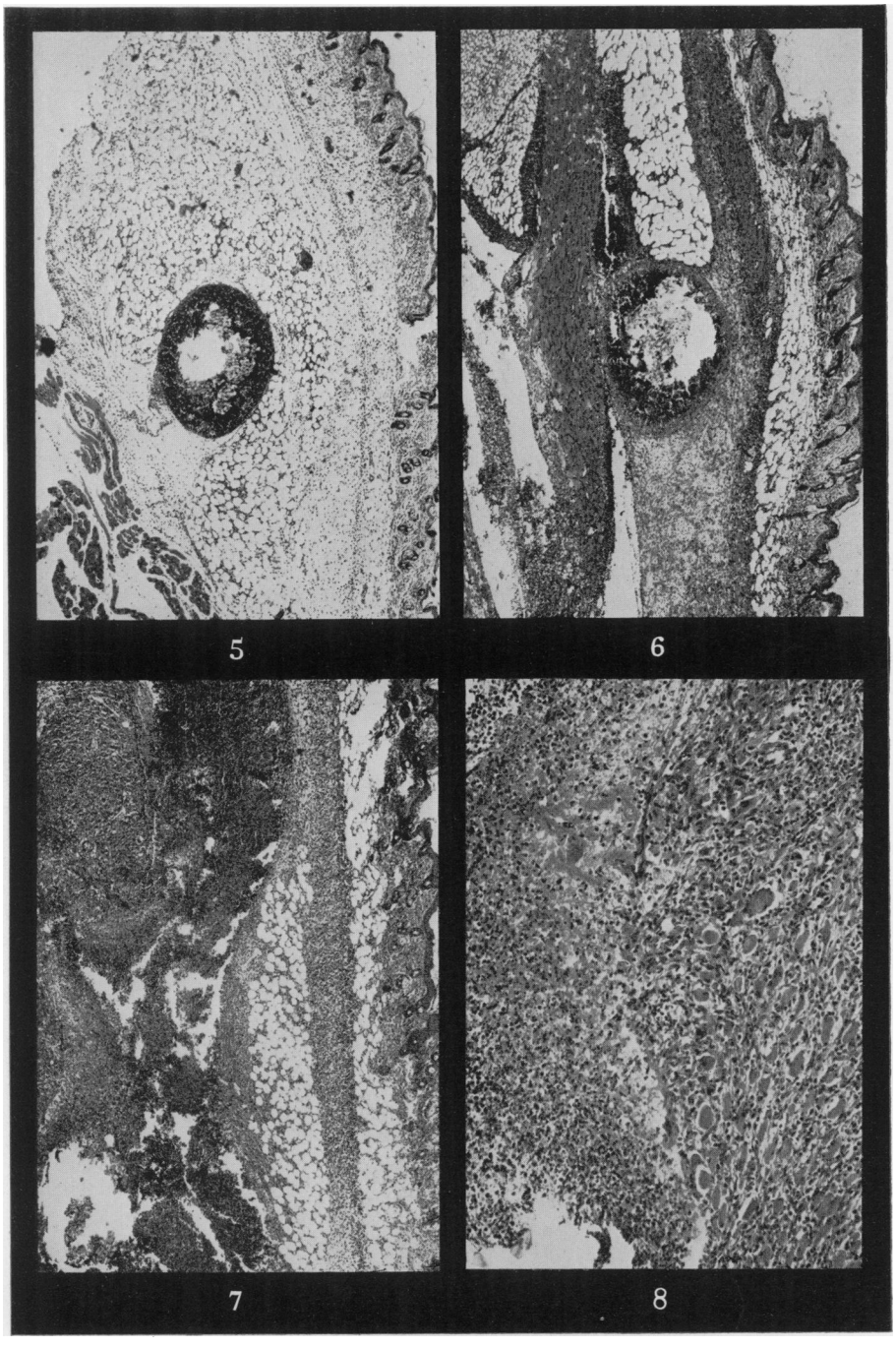

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Staphylococci of the phage type introduced on the suture were recovered from 16 spleens (4·3 per cent) ; 30 specimens of kidney tissue (8 per cent) ; 55 specimens of heart blood (15 per cent). One kidney abscess was found.

Preliminary experiments suggested that the smallest dose which uniformly induced pus formation was 1000–1500 coagulase-positive staphylococci. This dose was used for most of the early work.

TABLE II.—*Production of Abscesses by* 8 *Strains of Staphylococci of Phage Types* 80/81 *and* 52/80/81 *on Silk Sutures*

	Number of staphylococci per suture					
	10^6	10^5	10^4	10^3	10^2	10^1
Number of mice positive*	20	23	21	109	83	31
Number of mice negative	3	1	1	4	35	13
Per cent positive	87	96	95	96	71	70

* Positive = abscess with visible pus present at autopsy ; culture of pus yielded staphylococci.
Suture : 4–0 silk.

Table II summarizes the results of early experiments with 8 strains of phage type 80/81 or 52/80/81 from actively infected patients. The results confirmed the impression that 1000 organisms was the smallest uniformly effective dose. The data also show that the PFD_{50} (the dose resulting in pus formation in 50 per cent of the animals) was well below 100 organisms. If any difference in strain virulence were to be demonstrated by this method employing silk sutures, it would be necessary to use very small inocula of staphylococci, probably smaller than can be counted with reasonable accuracy by our method.

There is a further reservation that concerns the use of very small inocula. Sutures were not always retained subcutaneously but were sometimes lost within the first two days after insertion. When this happened infection might or might not follow. With small inocula relatively more sutures were lost and only 20–30 per cent of these sutureless mice developed infections. If the inoculum was 10^3 or higher, even when the suture was lost, infection followed in 50 per cent of the mice. Mice from which sutures were lost and which did not develop abscesses are not included in the totals shown in the various tables.

The LD_{50} by the intraperitoneal route for the strains used in the experiments summarized in Table II was 5×10^8 cocci, and the dose needed to cause pus formation by subcutaneous or intradermal injection was about 10^7 in the absence of a suture.

A limited survey of other coagulase-positive staphylococci introduced on silk sutures gave similar results, which are recorded in Table III.

The numbers of mice used to test individual strains were too small to draw conclusions about their relative virulence, but the PFD_{50} for the group as a whole was between 10 and 100 organisms. This was slightly higher than the PFD_{50} for the 80/81 strains, which appeared to be less than 10 organisms. However, the counting error at these low doses is unavoidably high and the difference may not be significant.

The same difficulty was encountered with lost sutures ; more were lost with small inocula and there were relatively more negative results among mice with missing sutures.

TABLE III.—*Production of Abscesses by* 14 *Strains of* Staph. pyogenes *other than Phage Types* 80/81 *or* 52/80/81

	Number of staphylococci per suture						
	10^6	10^5	10^4	10^3	10^2	10^1	<10
Number of mice positive*	31	66	68	201	103	36	3
Number of mice negative	3	1	2	16	76	33	19
Per cent positive	91	98	97	92	58	52	14

* Positive = abscess with visible pus present at autopsy ; culture of pus yielded staphylococci.
Suture : 4–0 silk.

In each experiment, 3–4 control mice were included and given 10^3 staphylococci of strain 5848 on a suture. This was a strain of phage type 52/80/81 which was originally isolated from a paronychia and had been used extensively in earlier experiments. All these mice, about 70 in number, developed abscesses, but they are not included in any of the totals because cultures were not taken from them.

Coagulase negative staphylococci.—Twenty coagulase negative strains were tested. These gave less consistent results than did the coagulase positive strains, not only because there appeared to be greater differences between strains, but also because single strains behaved differently at different times. It was much more difficult to predict the number of organisms a suture would pick up, but usually the counts were lower than those from similar initial concentrations of coagulase positive staphylococci.

Lost sutures were particularly troublesome when coagulase negative strains were tested in mice. However, 300 mice retained silk sutures bearing inocula ranging from 10^1–10^5 cocci. The results are summarized in Table IV.

TABLE IV.—*Failure of Coagulase Negative Staphylococci to Induce Abscess Formation*

	Number of staphylococci per suture				
	10^5	10^4	10^3	10^2	10^1
Number of mice positive	0	0	0	0	0
Number of mice " questionable "*	0	7	12	9	5
Number of mice negative	4	47	76	102	38

Cultures : 20 strains of coagulase negative staphylococci.
Suture : 4–0 silk.

* Mice recorded as " questionable " developed narrow tunnels of pus or necrotic material along the suture with no visible reaction in the surrounding tissue. Most of these were sterile on culture and staphylococci were recovered from the remainder, usually in small numbers.

None of the mice developed abscesses, but about one in eight was recorded as " questionable ". These questionable positives developed narrow "'tunnels " of pus or necrotic material along the line of the suture, with no visible reaction in the surrounding tissues. About half of the questionable positives were sterile on culture and staphylococci were isolated from the remainder, usually in small numbers. *Staph. pyogenes*, strain 5848 was again used as control ; 25 mice given 10^3 cocci of this strain all developed abscesses.

Histological reaction to sterile and infected silk sutures

The reaction to sterile silk 24 hr. after insertion was almost as extensive as the reaction to silk bearing coagulase positive staphylococci. There was a heavy polymorphonuclear cell infiltration and fibrin exudate immediately surrounding the suture, with oedema of the skin and subcutaneous tissues. The reaction to sterile silk remained relatively constant for about 2 days and then subsided.

The reaction to infected silk proceeded rapidly to central necrosis by the second or third day with great oedema of the surrounding tissues, and by the 5th day polymorphonuclear cell infiltration extended deep into the underlying muscle (Figs. 5–8).

Reinfection of mice recovered from original infection.—Twenty-six mice infected with 10^3 cocci of *Staph. pyogenes* strain 5848 on silk sutures were kept for 4 weeks, by which time all lesions had healed completely. Seventeen of these mice were then given the same dose of staphylococcus 5848 on silk sutures and the remainder received 10^3 organisms of a staphylococcus of Group II (phage type 3A/3B/3C/55/71).

All these mice developed large abscesses which appeared at about the same time as in previously uninfected mice and were comparable in size. When killed on the 6th day after infection they were found to have very much enlarged spleens with average weight of 350 mg., the largest weighing 700 mg. Spleens from 10 normal animals of the same age averaged 105 mg.

Cultures of the heart blood, the spleen and kidneys were made on all 26 animals as described in an earlier section of this paper. Heavy growths of staphylococci were recovered from 3 spleens, 8 specimens of kidney tissue, and 8 samples of heart blood.

Four colonies from each culture were phage typed. Mice in which second infections were caused by the Group II strain yielded colonies of the Group II type only. All cultures from mice given a second infection with staphylococcus 5848 were of the same phage type (52/80/81).

Cotton sutures

Cotton was used in one experiment only. It resembled silk in its ability to pick up staphylococci from broth, and in its capacity to initiate staphylococcal infection. Histological examinations were not made. It has been reported by Dettinger and Bowers (1957) that tissue reactions to silk and to cotton sutures resemble each other closely in type and extent.

Nylon sutures

Nylon sutures were used in several experiments. They proved much more difficult to handle than silk and one-fourth of the sutures were lost within 2 days of insertion. Counts of staphylococci were also much less reproducible and in general lower than those for silk, the highest obtained being 5×10^5 per suture. Early experiments suggested that nylon is very different from silk as a vehicle of infection and its use was continued in an attempt to establish this point. The results of these experiments are given in Table V.

There were a few questionable positives of the type seen with coagulase negative strains on silk, but most of the results were clear-cut negative or positive. The

TABLE V.—*Production of Abscesses by* Staph. pyogenes, *Phage Type* 52/80/81, *Strain* 5848, *on Nylon Sutures*

	Number of staphylococci per suture				
	10^5	10^4	10^3	10^2	10^1
Number of mice positive*	34	7	3	5	0
Number of mice negative	4	6	25	60	3
Per cent positive	90	55	11	8	—

Suture : 4–0 nylon.

* Positive = abscess with visible pus found at autopsy ; culture of pus yielded staphylococci.

PFD_{50} for staphylococcus 5848 on nylon appeared to be about 10^4, although the number of mice receiving this dose was unfortunately small (Table V).

The PFD_{50} for the same strain on silk was known to be less than 100 organisms. An attempt therefore was made to define the PFD_{50} for silk more exactly. The results are given in Table VI and show that the PFD_{50} is well below 100 organisms, but cannot be measured by our method because of the inaccuracy inherent in the enumeration of small numbers of bacteria.

The data in Tables V and VI do not permit detailed comparison of the infectivity of staphylococcus 5848 on nylon and on silk, but it seems probable that the PFD_{50} on nylon is at least 1000 times greater than the PFD_{50} on silk.

TABLE VI.—*Attempt to Determine the* PFD_{50} *of* Staph. pyogenes, *Phage Type* 52/80/81, *Strain* 5848, *on Silk Sutures*

	Number of staphylococci per suture			
	6×10^2	$9{\cdot}3 \times 10^1$	$3{\cdot}6 \times 10^1$	$2{\cdot}1 \times 10^1$
Number of mice positive*	20	9	11	10
Number of mice negative	0	5	4	4
Per cent positive	100	64	73	71

Suture : 4–0 silk.

* Positive = abscess, with visible pus found at autopsy ; culture of pus yielded staphylococci.

Histological reaction to sterile and infected nylon.—The tissue reaction to sterile nylon was considerably less marked than the reaction to sterile silk. At 24 hr. there was a fibrin reaction around the suture and moderate polymorphonuclear infiltration of the surrounding tissues. Slight inflammation was still present at 3 days but had almost completely subsided at 5 days, by which time there was a proliferation of fibroblasts and a few macrophages had appeared.

Nylon carrying small doses of coagulase positive staphylococci induced a more extensive reaction than sterile nylon and it took longer to subside. The reaction was still quite marked at 5 days and was surrounded by a wide zone of fibroblasts. The reaction to heavily infected nylon was indistinguishable from the reaction to infected silk.

Silicone-treated silk

It seemed possible that the superiority of silk and cotton over nylon as vehicles of infection was related to the wettability of the suture material. For this reason

silicone-treated silk was used. This material is said to be non-wettable, but retains the handling properties of untreated silk.

Silicone-treated silk was used in two experiments with staphylococcus 5848 and proved to be very similar to untreated silk in its ability to enhance infection. It also resembled silk in the number of organisms picked up from a given suspension of staphylococci and in yielding reproducible bacterial counts.

The results of experiments employing silicone-treated silk are recorded in Table VII.

TABLE VII.—*Production of Abscesses by* Staph. pyogenes, *Phage Type* 52/80/81, *Strain* 5848, *on Silicone-treated Silk*

	Number of staphylococci per suture			
	10^3	10^2	10^1	<10
Number of mice positive*	21	32	10	7
Number of mice negative	0	1	4	8
Per cent positive	100	97	71	47

Suture : 4–0 " Silicone Silk ".
* Positive = abscess, with visible pus found at autopsy ; culture of pus yielded staphylococci.

Histological reaction to sterile silicone-treated silk.—The tissue reaction to sterile silicone silk was much more extensive than the reaction to any of the other materials used. A heavy fibrin exudate formed around the suture and extensive polymorphonuclear infiltration of the surrounding tissues occurred. There was still a considerable amount of inflammation at 5 days but at this stage there were many macrophages and a fairly marked fibroblastic reaction.

Human hair

Staphylococcal infection is frequently associated with hair follicles, and it is also said that barbers are prone to develop purulent infections around fragments of hair which become imbedded in the skin of their hands, especially in the finger webs. These observations suggested that hair might be an effective agent for introducing staphylococci. Preliminary tests showed that staphylococci did not stick well to human hair. It was necessary to use concentrated suspensions to achieve inocula of more than 10^4 organisms. Doses from 10^2–10^5 cocci were finally introduced with results as shown in Table VIII.

TABLE VIII.—*Production of Abscesses by* Staph. pyogenes, *Phage Type* 52/80/81, *Strain* 5848, *on Human Hair*

	Number of staphylococci per suture			
	10^5	10^4	10^3	10^2
Number of mice positive*	2	1	0	0
Number of mice negative	18	8	9	8
Per cent positive	10	11	—	—

* Positive = abscess, with visible pus found at autopsy ; culture of pus yielded staphylococci.

From the results with human hair shown in Table VIII it can be seen that relatively high doses were needed to initiate infection and that hair was the least effective of the suture materials tried. However, hair appears to have a mild potentiating effect on staphylococcal infection in the mouse skin.

Histological reaction to sterile human hair.—The tissue reaction to sterile human hair was quite marked at 24 hr. There was a fairly heavy polymorphonuclear reaction and a fibrinous exudate around the hair, but by 3 days inflammation had almost completely subsided and at this stage it was much less marked than the reaction to any of the other materials used.

Dacron (Mersilene)

All the above suture materials appeared to enhance infection in rough proportion to the ability of the sterile material to induce an inflammatory response in the tissues. Dacron has been advocated as a suture material largely because the tissue reaction to it is said to be slight. It was used to test the suggested relationship between tissue reaction and infection. One experiment only was done, using staphylococcus 5848 and " Mersilene " suture material. The results are given in Table IX.

TABLE IX.—*Production of Abscesses by* Staph. pyogenes, *Phage Type* 52/80/81, *Strain* 5848, *on Dacron Sutures*

	Number of staphylococci per suture		
	$1{\cdot}4 \times 10^3$	$2{\cdot}1 \times 10^2$	<10
Number of mice positive*	8	9	4
Number of mice negative	0	0	3

Suture : 3–0 " Mersilene ".
* Positive = abscess with visible pus found at autopsy ; culture of pus yielded staphylococci.

This experiment is not strictly comparable to those where silk, nylon, and silicone silk were used, because at the time of the experiment the " Mersilene " suture was available only in 3–0 gauge which is slightly thicker than the other suture materials used. However, as can be seen from the results shown in Table IX, it seems likely that in its ability to enhance infection, dacron resembles silk and silicone-treated silk rather than nylon. Silk and dacron also resembled each other in their ability to pick up similar numbers of coagulase positive staphylococci from a suspension.

Histological reaction to sterile dacron sutures.—At 24 hr. the tissue reaction to sterile " Mersilene " was for the most part very similar to the reaction to nylon, but a few sections showed a more extensive reaction which almost equalled the effect of silicone-treated silk. Proliferation of fibroblasts and invasion by macrophages occurred earlier than with any of the other materials and by the 5th day these cells had largely replaced the acute inflammatory exudate.

Catgut

Catgut was used on one occasion only because the bacterial counts were very low and too variable to permit reasonable estimate of the dose used.

Steel wire

Steel wire picked up staphylococci very well and gave the most reproducible counts of any material used. Repeated shaking also showed that over 90 per cent of the organisms were removed at the first shaking and these counts were therefore probably more accurate than with any of the other materials. However, steel wire was found to be very difficult to handle. Thirty-five out of 40 mice lost their sutures within 24 hr. and for this reason its use was not continued.

DISCUSSION

The observations described demonstrate that subcutaneous abscesses can be produced consistently in mice by small numbers of coagulase positive staphylococci if the bacteria are introduced on certain suture materials, such as silk, silicone-treated silk, cotton, and dacron. All of these materials appear to be roughly comparable in their ability to potentiate infection by small numbers of staphylococci. With other suture materials such as nylon thread and human hair, much larger inocula of staphylococci were required. The potentiating effect of catgut and steel wire could not be tested adequately because of technical difficulties.

Among the 22 strains of coagulase positive staphylococci that were tested, it was not possible to distinguish differences in virulence, although with further experience this might be feasible.

Coagulase negative strains inserted on sutures uniformly failed to cause abscess formation. Great variability was found among coagulase negative strains and even among different cultures of the same strain, in their ability to be picked up and retained by the suture materials.

The effectiveness of the various suture materials in potentiating abscess formation when small inocula of coagulase positive staphylococci were used appears to depend upon two properties: their ability to pick up and retain staphylococci from broth cultures; and the severity of the polymorphonuclear inflammatory reaction caused by implantation of sterile sutures in mouse skin. For example, silk, silicone-treated silk, cotton and dacron, all highly effective as potentiators, are able to pick up about 10 times as many staphylococci per unit length as nylon. Human hair was even less effective than nylon in picking up the cocci. Furthermore, sterile silk, silicone-treated silk and dacron, all of which are effective potentiators, produce a marked and persistent polymorphonuclear inflammatory reaction in mouse skin, whereas nylon and human hair, which are weak potentiators, cause a much milder inflammatory response.

With the more effective suture materials, introduction of approximately 10^3 coagulase positive staphylococci caused abscess formation in almost all mice. Attempts to define the number of cocci that would induce abscess formation in 50 per cent of the animals were only partially successful because the methods do not provide a good estimate of inocula of less than 100 cocci. The errors involved in recovering small numbers of staphylococci from the sutures are great and they are exaggerated by the difficulties inherent in counting small numbers of bacteria. Sutures carrying small inocula are also more liable to be lost than those carrying larger doses. However, if the suture is retained, abscess formation takes place in a large proportion of animals given an inoculum of 10–100 staphylococci.

After introduction of contaminated silk sutures and other materials that act similarly, abscess formation was usually apparent within 4–5 days. At this time cultures of the blood, spleen and kidneys were found to be positive in a small percentage of the animals. If the abscess ruptured, which was common early in the course of the infection, complete recovery occurred promptly. If the abscess did not rupture, healing was much slower, but in most cases was complete in about 4–5 weeks. At this time reinfection at the same site with the same dose of the homologous strain or one of a different phage type, resulted in the development of abscesses which did not differ significantly from those seen at the first infection. Moreover, blood and organ cultures were commonly positive in reinfected mice. It would appear, therefore, that if immunity develops in association with recovery from the primary infection, it is slight in degree, because it provided no demonstrable protection against reinfection under the conditions employed. However, it is possible that immunity might be demonstrable if smaller inocula were used for reinfection.

With respect to immunity it should be noted that staphylococcal infections of the skin in man are characterized by a remittent course with multiple episodes of furunculosis that commonly recur over periods of months to years. Intervals of relative or complete freedom from overt lesions often appear, but there is little evidence that recovery from one bout confers immunity against a second, even though the infecting staphylococci belong to the same phage type on both occasions. If anything, the reverse seems to be the case, because a person who has recovered from an attack seems to be more susceptible than normal to staphylococcal infection.

The method described appears to possess some of the features required of an experimental model of human staphylococcal infections. In particular its simplicity, reproducibility, the use of small inocula and the production of an easily observable, superficial lesion, commend its trial in the investigation of staphylococcal disease. Materials other than those studied might be found which would be more suitable especially for the induction of infections with less than 100 cocci. For example, a suture of some soluble substance such as calcium alginate would permit more accurate estimation of dose.

Although our results show that various suture materials differ greatly in their ability to potentiate staphylococcal infections in young mice, it should not be concluded that these differences pertain also to the use of the same sutures in man. Elek (1957) has demonstrated that silk thread has a striking effect in reducing the inoculum of staphylococci needed to cause abscesses in the skin of human volunteers. The present studies show that silk and certain other materials behave similarly in young mice. On the other hand, preliminary experiments indicate that nylon sutures, which require a relatively large inoculum of staphylococci in young mice, can cause abscesses with much smaller inocula when older mice are used.

It is tempting to draw conclusions from the resemblance of these experimental infections to many human staphylococcal infections. The gross and histological appearances of the lesions, their evolution, and the ease with which mice can be reinfected, all suggest analogies with superficial staphylococcal infection in man. However, it remains to be seen whether any valid comparisons can be made and whether this method can provide information on the course of staphylococcal infection in any animal other than the mouse.

SUMMARY

Local skin abscesses have been induced in young mice employing small inocula of various strains of *Staph. pyogenes* introduced on a number of suture materials.

Differences in virulence among various strains of coagulase positive staphylococci were not apparent.

Coagulase negative strains of staphylococci did not give rise to local abscesses.

Of the sutures used, silk, silicone-treated silk, cotton and dacron were most effective in potentiating infection. Nylon and human hair were less effective in that they required larger inocula of staphylococci.

The potentiating effect of the suture materials appeared to be related to the severity of the polymorphonuclear inflammatory reaction which the sterile materials caused in the mouse skin and to the capacity of the sutures to pick up staphylococci from broth cultures.

Mice that had recovered from one infection showed no evidence of increased resistance upon reinfection with homologous or heterologous strains.

We wish to thank Dr. Ashton Morrison of the Department of Pathology for his generous assistance in preparation of the material for histological examination and in its interpretation.

We are grateful to Mrs. Elizabeth Breisch and Mrs. Laura Cohn for their expert technical assistance.

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