Experimental staphylococcal infection in humans

R. R. Marples

Considerable insight can be derived from experimental staphylococcal infections in animals. However, human skin differs from animal skin in certain important respects, making it necessary to extend experimentation to man. Many of the experiments described in the literature were designed to prove that a particular form of lesion was caused by the staphylococcus rather than to investigate pathogenic mechanisms, assess virulence, or appraise treatments.

Only a few years after Ogston (1881) had demonstrated that acute abscesses contained staphylococci and that pus was infectious to animals, man was employed as an experimental animal. Garré (1885) was the first; but his first attempt failed and for his second experiment he rubbed an entire culture into the skin of his left forearm. Small pustules developed over the next 12 hours which coalesced into 'eine grosse furunkel', a carbuncle which healed with scarring over a period of three weeks.

Bockhart (1887) performed several experiments upon himself. His main interest was in impetigo. He could see monococci and diplococci in the pus from lesions and could culture *Staphylococcus pyogenes aureus* and *albus* sometimes alone but usually together. The inoculum he used for his experiments was therefore a mixture of cultures of *S. aureus* and *S. albus*. His description of his experiments was very detailed and it is possible to learn from his work. On 14 April 1886 at 4 p.m. he inoculated a site on the outside of his left forearm which had been cleaned and disinfected and the horny layer lightly scraped away with a scalpel. By 10 p.m. the site was slightly reddened and somewhat painful and by 6 a.m. on the next day 25 tiny pustules were visible only one of which was pierced by a hair. The pustules dried up over the next 6 days without scarring. The contents of 8 pustules were examined microscopically and cultured on day 2. Cocci were visible in the pus and both *S. aureus* and *S. albus* were cultured. A biopsy was taken on day 3 which showed a folliculitis.

Encouraged, he performed a second trial on 25 April. After disinfecting the skin he scratched an area with his fingernail and rubbed in the inoculum. After 12 hours 35 pustules appeared at the site and others beneath his eye. Most pustules regressed but two progressed to a painful furuncle and lesions continued to appear at the inoculated site for 3 months. Bockhart was also successful in producing infection by intradermal injection of a mixed culture into his finger but recovered only *S. aureus* from the abscess.

The experiments of subsequent investigators involving the injection of cultures or pus into the skin were successful in producing lesions showing a variety of clinical appearances (Neisser, 1928) but inunction into normal skin gave contradictory results. Epstein (1940) answered the criticism that experimental lesions were not bullous impetigo by publishing a photograph of an experimentally produced bullous lesion which continued to show a vesicular character for 19 days after inoculation of a pure culture of *S. aureus* obtained from a lesion of impetigo.

The interest in impetigo continued because many workers claimed that streptococci were the cause of the disease and it is not until relatively recent times that the clinical distinctions between staphylococcal impetigo and streptococcal impetigo have been accepted. This was not so in 1943 when two papers appeared together in the *Lancet*. Bigger & Hodgson (1943) studied 130 patients and isolated staphylococci from 124 and streptococci from 50. They made many attempts to transmit impetigo using crusts, fluid from oozing lesions and vesicles as well as suspensions of strains of *S. aureus* isolated from cases of impetigo. Infections occurred infrequently when the skin was subjected to scratching, scarification or multiple puncture and the inoculated site covered with a sterile dressing held in place with elastoplast, and not at all with less traumatic procedures. Only the intradermal injection of vesicle fluid or a suspension of *S. aureus* invariably produced an infection. The authors were not convinced that any of the lesions, which ranged from erythema to pustules, could be called impetigo while Sheehan & Fergusson (1943) were sure that they had reproduced the disease. Again these workers were unsuccessful when the inoculum was rubbed into undamaged skin. Their final experimental model included thorough scarification of an area 1 cm in diameter sufficient to allow some oozing of blood and an inoculum of a large loopful of culture from blood agar or of fluid from a fresh vesicle. After inoculation the sites were covered with a metal cap held in position by sticking plaster. All 18 inoculations of vesicle fluid and 11 of 13 inoculations of *S. aureus* produced lesions, but in only 4 of each, was the lesion typical of impetigo, the remainder being less severe. No infections followed inoculation of *S. albus* and only a transient

papular response was seen in 7 of the 9 inoculations of *Streptococcus haemolyticus*.

The first application of experimental infections in man to the assessment of virulence and the comparison between strains of *S. aureus* was that of Elek & Conen (1956, 1957). They applied the concept of the minimum pus-forming dose used by Panton & Valentine (1928) for rabbits, to human experimental infections. The minimum pus-forming dose by intradermal injection was found to be 2 to 8 million organisms in a volume of 0.1 ml and no less for subcutaneous injection and full thickness skin incisions. The dose was not reduced by additional preformed toxin and no difference was found between strains isolated from healthy nasal carriers and strains from recent human lesions.

The most dramatic finding was the effect of a foreign body. Three silk sutures carrying 3×10^4 cocci were inserted through the skin and subcutaneous tissue in 2 volunteers. One was pulled through, one tied at normal tension and the third tied tightly. The two volunteers became very ill within 24 h and the sutures had to be removed. The site where the suture had been pulled through showed no reaction although 4/5 of the inoculum was left in the skin, while the other two sites developed large stitch abscesses which took a week to resolve even on penicillin therapy. No observable difference between standard and tight sutures could be seen though the volunteers stated that the tight sutures were more painful. Volunteers became difficult to find but in one volunteer, 10^2 cocci on a suture produced a small stitch abscess, an enhancement of virulence of four orders of magnitude.

A return to the scraping method of preparing the experimental site, inoculating with a phage group II strain from a wound and covering the site with a glass coverslip held in place with cellophane tape was described by Foster and Hutt (1960). The effect of protection from desiccation was shown by counting the cells recovered from the lesion and coverslip—on the sealed lesions the count rose from 0.7×10^6 to 18×10^6 in 8 h during which time the exposed lesions had shown no increase in numbers. In their system *S. albus* was able to produce infection but the inflammatory response was less than in the lesion infected by *S. aureus*. The smallest dose of *S. aureus* causing a seropurulent exudate was 15 cells. Two of the volunteers developed boils on the inoculated arm and, in one case, abscesses on the thigh and buttock from which *S. aureus* of the same phage type as the test strain was cultured, ending the series of experiments.

The difficulties of producing an experimental lesion that was tolerable to the volunteer yet could be accepted as a true infection were emphasized by Maibach (1965). He reported on the results of over 1000 inoculations with only one untoward infection. Inoculation of normal skin was unsuccessful

and pretreatment with ultraviolet light did not improve the success rate. It is not clear whether removal of the outer horny layer by cellophane tape stripping or sandpapering or by scalpel really predisposed to infection. Exudates, even when produced, do not seem to have been cultured and the reaction was merely compared to a control site. The possibility of cross infection was not excluded. Following Foster and Hutts' (1960) technique Maibach made 500 inoculations in 40 subjects on abraded skin without ever seeing the development of a greater seropurulent exudate than seen at the non-inoculated control site. When plastic covers filled with agar seeded with the test strain were applied a few pustules developed usually under the plastic rim. Again intradermal injection of more than a million organisms was needed to produce pus and in one instance, intradermal injection of the thigh was followed by a deep infection of the arm which resolved slowly under penicillin therapy. Maibach comments that as studies became more extensive so the infection rate fell.

Duncan, McBride and Knox (1970) also used the agar-filled cup method, first introduced by O'Brien (1950), with partial success with *S. aureus* in 10 of 78 attempts but only one in 78 attempts with *Streptococcus pyogenes.* Multiple methods of traumatizing the skin were then employed with only 2 infections out of 35 attempts, and these following inoculation of mixed cultures of *S. aureus* and *Strep. pyogenes.* When lancet stab wounds were rubbed with polyethylene granules 6 infections were produced after inoculation of *S. aureus* and *Strep. pyogenes* in the first 10 trials but no further infections occurred in a total of 180 trials. Moving the experimental area to the legs improved the frequency of experimental infection from 14 per cent to 37 per cent.

Conclusions from the historical accounts

It seems adequately proven that *S. aureus* can cause disease but the conditions that must be provided for experimental infections are severe. Although the defences of most can be overwhelmed if the inoculum is large enough several other factors can be noted. Mild trauma to the skin—a Rebuck skin window (Rebuck & Yates 1954)—seems a more suitable preparation of the site than implanting the organisms into the skin by intradermal injection. A foreign body is also able to promote infection at a dose of organisms that is credible. Successful investigators who have not used massive inocula seem to have protected the site to prevent it drying, a procedure that may be critical in experimental infections.

When I joined the Philadelphia school, experimental infections in man were felt to depend on two factors. One was true occlusion of the skin and

the other was that the dose of organisms inoculated should be as small as necessary. Quantification was deemed important.

S. AUREUS INFECTIONS ON INTACT SKIN

The results of experiments aimed to elucidate the role of infection in miliaria suggested that the normal flora of the skin was of considerable importance in regulating the growth of potential pathogens. In order to delineate the role of bacteria in anhidrosis after the application of occlusive dressings to the skin, dressings of different sizes were placed on sites treated or untreated with neomycin. When dressings of 5 cm^2 were applied to normal forearm skin, neomycin treatment led to overgrowth of yeasts. When large dressings occluded the whole forearm in an environment where neomycin was widely used, and neomycin was applied to the skin, selection and overgrowth of neomycin resistant *S. aureus* occurred in 7 of 10 subjects. After one week the volunteers developed a toxic pyoderma (Marples & Kligman 1969) while the control sites on the opposite arm showed no signs of infection. This accidental experimental infection suggested that trauma was not absolutely necessary for the induction of infection by *S. aureus* provided that competition from the normal flora was reduced and a fully hydrated micro-environment was maintained.

The aim of the first series of experiments was to induce cutaneous lesions by growing *S. aureus* on undamaged skin (Singh, Marples & Kligman 1971). The test organism, usually an antibiotic-susceptible non-typable strain of *S. aureus* isolated from a skin lesion, was suspended in saline in about 10^9 cfu per ml and diluted appropriately. The viable count was determined each time. The site to be inoculated was prepared by placing a gauze pad saturated with 70 per cent ethanol on the volar forearm for 2 min. After the skin had dried, the inoculum, in a volume of 0.02 ml, was placed on the skin and immediately covered with 3 cm^2 of plastic film which distributed the inoculum evenly over the site by capillarity. The dressing was firmly fastened to the skin with zinc oxide adhesive tape which is antibacterial, preventing spread of the infection (Marples & Kligman 1969). The dressings remained in place up to 6 days. Quantitative samples were taken by the detergent-scrub method (Williamson & Kligman 1965). At the end of each experiment the inoculated site was treated topically for 2 days with neomycin– polymyxin–bacitracin cream.

It could be shown that alcohol pretreatment reduced tenfold the dose

required to infect half the sites (ED_{50}). In 20 volunteers the ED_{50} was about 10^3 cells per cm^2 but infections were produced in some sites for each of the six dose levels tested on each volunteer. In further experiments, multiple sites were inoculated with the same number of viable cells and samples were taken at different times to establish the population kinetics of the system. Both in the volunteers that subsequently displayed lesions and those that did not, the number of cells recoverable fell during the first 24 h by one order of magnitude. This fall continued in the 'uninfected' group with only a few viable cells of the inoculated strain to be found at 4 days. In the other group the inoculated strain started to multiply after 24 h, increasing about tenfold daily, until about 10^7 cells/cm^2 was reached. About 2 days later the first signs of redness and a papular rash developed which evolved into pustules. With an initial dose of 2×10^7 cells this development was complete in 6 days but took longer with lower doses.

Histological examination showed spongiosis of the epidermis and infiltration with polymorphonuclear leucocytes and lymphocytes. Subcorneal vesicles and pustules developed and finally focal necrosis could sometimes be seen. There was no evidence of invasion of the tissue by bacteria and the conclusion was again that a toxic pyoderma had been induced similar to the biological type of contact dermatitis described following experimental human infection with *Candida albicans* (Maibach & Kligman 1962, Rebora, Marples & Kligman 1973).

S. aureus Infections following Skin Trauma

The aim was to create a reproducible superficial trauma for which the method of stripping off the horny layer with cellophane tape was selected. This procedure does more than remove the horny layer, it damages the epidermis provoking inflammation and exudation of fluid onto the surface. However, the residual epidermis is continuous and leucocytes do not appear in the exudate in the absence of infection.

The experimental 2 cm^2 sites were stripped off to the glistening layer. After a delay of 24 h 0.01 ml of inoculum (usually 10^3–10^5 cfu) was applied and covered with a 2 cm^2 plastic film held in place with the same adhesive tape as before for 24 h, after which quantitative bacteriological samples were taken. The exudate on the plastic film was transferred onto a clean slide and stained with May Grunewald Giemsa. The clinical severity of infection was graded on a 5 point scale (Marples & Kligman, 1972).

Characteristic clinical appearances were a visible, sometimes copious,

exudate, usually serosanguineous but sometimes frankly purulent. The base of the lesion was bright red and sometimes the residual epidermis was eroded. The site was tender but without much swelling. After removal of the dressings the sites healed rapidly but because of a few exceptions antibiotic cream was applied twice daily for 2 days as a routine.

The exudate usually showed neutrophils and cocci. When copious, very few bacteria were seen though bacteriologically most sites yielded 10^8 cfu. These findings were taken to indicate infection. When inoculation was delayed for 48 h many organisms and very few leucocytes were seen. These findings were interpreted as colonization only.

Inoculation, immediately after preparation of the site, of 10^5 cells was performed in 10 volunteers. Within 6 h two subjects complained of pain and swelling and there were signs of cellulitis in all the volunteers. The experiment was ended, all subjects received systemic antibiotics and there was rapid regression of the signs and symptoms.

Inoculation 48 h after stripping caused fewer infections than after 24 h; only 5 of 42 sites inoculated after 48 h became infected while 15 of 21 sites inoculated after 24 h did so. The experiment to determine the ED_{50} was carried out after a 48 h delay. Most sites became heavily colonized at all doses of 400 or more, while only 4 of 12 sites inoculated with approximately 10 cells were heavily colonized. The numbers of organisms recovered from colonized sites were very similar despite differences in inoculum, as Foster & Hutt (1960) had found.

The model was used to test the activity of antistaphylococcal and antifungal antibiotics in combination with a fluorinated corticosteroid (Marples, Rebora & Kligman, 1973). Inoculation was performed 24 h after stripping; the sites were covered with an occlusive dressing for 6 h before the treatments were applied and the dressings replaced for a further 18 h when quantitative samples were taken. The results showed the expected reduction in final count where the antibiotics were present but the steroid did not enhance growth nor lead to untoward effects. An objective appraisal of the various formulations was possible.

Summary and Conclusions

The problem with experimental *S. aureus* infections in man is to find a balance between failure to produce any infection and too severe an infection. Intradermal injection has been the most reliable route (Neisser 1928) but a large dose of 2×10^6 organisms in 0.1 ml seems to be necessary (Elek & Conen, 1957). Smaller, more credible numbers can produce lesions

only in special circumstances where the organisms are protected from efficient phagocytosis by the presence of a foreign body such as a silk suture (Elek & Conen, 1957) or by damage to, but not penetration of, the epidermis and measures to prevent the inoculum drying up. Scarification (Sheehan & Fergusson, 1943), scraping with a scalpel, and in addition, covering the site with a coverslip (Foster & Hutt, 1960), or stripping and plastic film occlusive dressings (Marples & Kligman, 1972) have given infections from small inocula. The degree of trauma seems to be critical, for Elek & Conen (1957) found that similar doses to those required to form pus after intradermal injection were required to infect full thickness incisions.

It is surprising how many investigators have tried with so little success to reproduce lesions in intact skin by rubbing in large numbers of living cells either in the form of lesion fluid or in pure culture. Maibach (1965) summarizes this list of authors and reported on over 1000 experiments. Singh, Marples & Kligman (1971) could produce a toxic pyoderma reliably only by protecting the site, removing competitors, maintaining the dressings in place for prolonged periods and using high doses of *S. aureus*. The reasons for attempting experimental infections in humans have changed. The early work was designed to prove Ogston correct and that *S. aureus* was the cause of human disease. Garré's 'grosse furunkel' did that. Later a controversy over a staphylococcal or a streptococcal aetiology of impetigo contagiosa engendered several attempts to reproduce this disease experimentally with some success (Epstein 1940, Sheehan & Fergusson, 1943) but sometimes with doubt about the true nature of the experimental lesion (Bigger & Hodgson, 1943). Elek (1956) was interested in detecting differences in virulence between strains from lesions or from carrier sites. No difference could be detected, a result confirmed by Singh, Marples & Kligman (1971). The Philadelphia school (op. cit.) and Duncan, McBride and Knox (1970) were interested in developing a reproducible method in order to test the efficacy of antibacterial formulations, as well as to develop an explanation of the initial stages of infection. The importance of immunological status seems to be overlooked in the modern work but Bockhart's results (1887) and perhaps those of Foster & Hutt (1960) show increasing severity of the infections rather like those described in the rabbit (Panton & Valentine, 1929).

Awareness of the danger to health, adverse publicity and ethical considerations have combined to restrict experimental infections to those truly interested in the result. It should remain that way.

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