THE PREVENTION OF EXPERIMENTAL *CLOSTRIDIUM NOVI* GAS GANGRENE IN HIGH-VELOCITY MISSILE WOUNDS BY PASSIVE IMMUNISATION

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PLATES XXIV–XXVI

GAS-GANGRENE infection is caused by the contamination of wounds and the growth therein of three species of clostridia, viz. *Clostridium perfringens* type A, *Cl. novyi* types A and B and *Cl. septicum*, separately or in combination. These organisms produce enzymes that assist their massive and rapidly spreading growth in devitalised tissue; they also produce necrotising and haemolytic exotoxins that have lethal activities.

Clostridial infection following high-velocity missile injuries to limbs has been responsible for much morbidity and mortality in various military conflicts. Characteristic of these wounds is the large area of dead and dying tissue which provides ideal conditions for the anaerobic multiplication of clostridia. The surgery of limb wounds is especially directed at removing this tissue in an attempt to prevent such infection. The effectiveness of surgery is greatly influenced by the time elapsing between wounding and treatment. It is not always possible for casualties in forward military areas to be promptly evacuated and to receive early definitive treatment. Accordingly, the present work was undertaken to assess the effectiveness of antitoxin prophylaxis against *Cl. novyi* infection in the light of results obtained with an experimental sheep model developed to simulate war wounds.

Opinion is divided on the value of passive immunisation for the prevention of gas gangrene in man. Jeffrey and Thomson (1944) felt that antitoxin did little to affect the outcome of the disease. In 33 cases of gas gangrene following war wounds, 12 died in spite of surgery, antitoxin and penicillin. Ten of the 33 cases had received prophylactic antiserum on admission, and of these, four died. The antiserum was a mixed one containing antitoxins to the alpha toxins of *Cl. perfringens*, *Cl. novyi* and *Cl. septicum*. MacLennan (1943) on the other hand, reviewing the treatment of gas gangrene during the Middle East conflict, found that although sulphonamides were ineffective, the recovery rate after the administration of antitoxin was 75 per cent. in 66 cases. In groups that received only sulphonamide (28 cases) or no specific treatment (19 cases), the survival rate was 21 per cent. MacFarlane (1945) administered large therapeutic doses of polyvalent antitoxin to patients after the disease had been diagnosed. He demonstrated that while radical surgery was clearly responsible for a significant lowering of the death rate to 47 per cent., the combination of prompt intravenous antitoxin therapy with surgery was even more beneficial (death rate 20.6 per cent.).

Received 15 Jan. 1972, accepted 30 Mar. 1972.

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The therapeutic value of antiserum has been extensively investigated in animals. Hartley and Evans (1945) and Stammers (1946) were in no doubt that antitoxin was beneficial in the protection of animals against an experimental challenge with *Cl. perfringens* alpha toxin, and Hall (1945) reached similar conclusions from an experimental evaluation of a bivalent and a pentavalent antiserum. Owen-Smith (1968) gave gas-gangrene antiserum prophylactically to contaminated wounded sheep and demonstrated a possible relationship between the interval between wounding and antitoxin administration and its prophylactic value.

In the investigation of gas gangrene following high-velocity missile wounds, the sheep has proved to be a satisfactory experimental animal (Owen-Smith, 1968; Owen-Smith and Matheson, 1968). In such wounds, tissue destruction is chiefly caused by a process known as cavitation. The damage surrounding a missile track in muscle may be extensive, the ischaemic myonecrosis providing the medium upon which the gas-gangrene species multiply. It has been shown by Thoresby and Darlow (1967) that wound contamination in any high-velocity missile injury occurs chiefly by the sucking in of surface organisms at the entrance or exit wounds. Other debris such as clothing and dirt is also found in the depths of the wound, carried in not only by the missile but also by the suction effect that accompanies the cavitation phenomenon seen in this type of injury.

Hopkinson and Watts (1963) established a standard experimental muscle wound which has been modified in the present work in order to ensure almost 100 per cent. mortality from gas gangrene in the control groups. The protective effect of a mixed gas-gangrene antitoxin in preventing the disease when missile wounds in sheep were contaminated with *Cl. novyi* type-A spores is studied with special reference to the time interval between wounding and treatment.

MATERIALS AND METHODS

Animals. Ewes aged 1–2 yr with no history of immunisation against *Cl. novyi* and weighing between 40–70 kg were starved for 24 hr before anaesthesia; water was allowed up to 6 hr before the challenge.

Anaesthesia. Anaesthesia was induced by the intravenous injection of 6 per cent. sodium pentobarbitone, 10 mg per kg body weight, usually into the jugular vein. Anaesthesia was maintained with nitrous oxide and oxygen if the surgical procedures exceeded about 15 min.

Wounding. The sheep was placed on the operating table and an endotracheal tube was passed to facilitate breathing and to cope with the excessive salivation that was not controlled by atropine. One hind limb of the sheep was shaved and suspended from a stand. The adductor group of muscles was selected as the target area, marked with red adhesive tape and aligned with the fixed sights of a rifle. This alignment had to be done accurately, taking into account the position of the femur and the femoral artery. The target area was covered with a piece of battledress cloth c. 50 cm² in the centre of which had been absorbed 1 ml of a wet suspension of spores of *Cl. novyi* type A containing 250,000 per ml. The cloth was contaminated 15 min. before challenge, the effective area being a damp spot 1–2 cm in diameter. The missile was an 0.22 cm Hornet bullet with a reduced velocity of 2000 ft per second. The rifle was fired electrically from an adjacent room in order to avoid the danger to personnel by ricochet and the bullet was made to pass through the centre of the battledress and then through the thigh. The suction that accompanied these cavitation wounds caused spores from the battledress to enter deeply into the wound. Only a small piece, seldom greater than twice the diameter of the bullet, was missing from the cloth after challenge. This had been carried into the wound and was sometimes seen at necropsy. There was little bleeding from the wound.

Post-injury care. The wound was bandaged and each sheep was returned to its pen with the endotracheal tube still *in situ*. The animals were placed prone to avoid chest complications due to the weight of the intestine on the diaphragm. When they had regained consciousness and had started to chew, the tube was removed. Within 2 hr after injury, the sheep were

moving in their pens. They were kept under observation for 48 hr and the surviving fit sheep were then let out to pasture.

Challenge spore suspension. The Jolly strain of *Cl. novyi* type A used was originally isolated from a soldier of that name who had gas-gangrene infection whilst on active service in the Western Desert of North Africa. A suspension of the spores in distilled water was kindly supplied by Dr Keppie of the Microbiological Research Establishment, Porton, England, and was stored at 4°C. Spore counts were carried out with a Neubauer chamber and suspensions were heated to 60°C for 1 hr before use. Sheep were challenged in groups of up to eight; one or two in each group did not receive antitoxin therapy and served as controls.

Antitoxin. The antitoxin used for passive protection was Mixed Gas Gangrene Antitoxin B.P. (Burroughs Wellcome & Co. Ltd). It contained 10,000 units *Cl. perfringens* alpha antitoxin, 5000 units *Cl. septicum* alpha antitoxin and 10,000 units *Cl. novyi* alpha antitoxin in each 10-ml phial. Each dose consisted of the contents of one phial given intramuscularly into the semispinalis muscle. The first dose was given at various times after challenge and then 12-hourly for a further 48 hr. In the group given antitoxin 1 hr after challenge, no further doses were given.

Post-mortem investigations

Negative controls. Fifteen sheep were wounded but not intentionally contaminated with *Cl. novyi* spores and killed and subjected to necropsy 1 mth after injury in order to determine the effect of wounding and to confirm the absence of extraneous contamination with *Cl. novyi*.

Positive controls. Control sheep, i.e., those that had been contaminated during wounding but which did not receive antitoxin therapy, were examined macroscopically and microscopically after death. Sheep that survived the challenge because of antitoxin therapy were usually killed 3 wk later and examined.

Bacteriology. Muscle tissue taken from the wound track was incubated in Robertson's meat broth. Blood agar plates were seeded from this broth after 48 hr and incubated in an anaerobic jar for 48 hr. Biochemical tests including sugar fermentation and the Nagler reaction for phospholipase were performed. Muscle tissue smears were examined microscopically by the Gram stain and by the fluorescent antibody (FA) technique for the specific identification of *Cl. novyi* (Batty and Walker, 1964).

Histology. Muscle tissue was taken from the vicinity of all wound tracks. After fixation for 48 hr in 10 per cent. formol saline, sections (6–8 µm) were cut from paraffin blocks, stained with haematoxylin and eosin and examined microscopically.

RESULTS

Sheep not intentionally contaminated with gas-gangrene organisms during injury (negative controls) survived without apparent ill effect. Out of a total of 15 sheep, three developed abscesses in the challenged leg. There was a mixed growth of organisms from these abscesses. All sheep at necropsy 1 mth after injury had developed a fibrous track.

Controls that had been contaminated but had received no antitoxin therapy (positive controls) numbered 68. Of these, 62 died of *Cl. novyi* gas gangrene within 72 hr. There was one anaesthetic death and the remaining five animals survived. The cause of death was confirmed by macroscopic, histological and bacteriological examination.

Several experimental groups of animals were used to determine the effectiveness of gas-gangrene antitoxin after contamination. The results obtained are shown in the table. Only in those groups given antitoxin 1 hr after contamination was there 100 per cent. survival. The gas-gangrene deaths that occurred

were typical with much myonecrosis. *Cl. novyi* was present in the missile track and the peripheral muscle. Of those animals that died of causes other than gas gangrene, two were anaesthetic deaths, one was due to massive haemorrhage associated with a large exit wound and the fourth was due to a fractured femur. There was no evidence of *Cl. novyi* in any of these sheep.

Pathology associated with gas gangrene

Animals dying of *Cl. novyi* gas gangrene had a sickly smell. The infected limb was swollen and hot, the skin had a purple tinge and there was a sero-sanguinous discharge from the wound. The infected muscle was spongy, the bundles being separated by gas, and the tissue colour ranged from bright red

TABLE

The effect of the time interval between wounding and the administration of gas-gangrene antitoxin on the survival rate of sheep challenged at wounding with spores of Cl. novyi type A

Time between wounding and administration of antitoxin (hr)	Number of sheep wounded	Number of deaths attributable to		Number of survivors at 200 hr
		gas gangrene	other causes	
1	11	0	0	11
9	105	3	3	99
12	18	1	1	16
15	12	1	0	11
18	8	2	0	6
21	4	4	0	0

to dark purple (fig. 1). One characteristic feature was the presence of sub-cutaneous gelatinous oedema. Muscle taken from the vicinity of a wound track showed eosinophilic myonecrosis with fragmentation of the fibres separated by oedema fluid. Many of the muscle fibres were structureless, the nuclei demonstrating karyorrhexis or karyolysis (fig. 5). There was very little polymorphonuclear leucocyte infiltration.

A smear of muscle tissue taken from a control animal that died 48 hr after contamination shows organisms scattered throughout the spaces separating the muscle bundles (fig. 6). The bacteria were Gram-positive and arranged singly, in pairs or in chains. These organisms fluoresced brightly when stained with fluorescently labelled *Cl. novyi* antiserum.

Pathology in a wound associated with gas-gangrene antiserum prophylaxis

In the challenged sheep that were protected by antitoxin therapy and survived, a large thick-walled abscess characteristically developed within the adductor muscle mass (fig. 2). The abscess was well formed 10 days after challenge. The hind leg of the sheep was swollen and the animal walked with a slight limp. Three to 6 wk after challenge the abscess had usually pointed

along the bullet track to the exit wound (figs. 3 and 4); and by 12 wk it had frequently burst and pus was extruded. Three months after injury there usually remained only a fibrous track. The sheep no longer limped. Histological section of the abscess cavity and surrounding wall demonstrated a pyogenic reaction and the cavity contained the ghosts of dead muscle fibres. There was an acute polymorphonuclear infiltration round the dead muscle (fig. 7). Peripheral to the polymorph infiltration were fibroblasts in varying stages of maturity.

Bacteriological examination of the abscess cavities of sheep surviving challenge as a result of antiserum therapy showed the presence of healthy vegetative organisms of *Cl. novyi* that could be cultured. Organisms could still be demonstrated by the FA technique in the abscesses of sheep killed 80 days after wounding. However, in contrast to the brightly staining organisms observed in the smears from typical cases of gas gangrene, the organisms were elongated and poorly stained. Cultures from such abscesses were occasionally positive. By 90 days, all the abscesses had drained and no evidence of *Cl. novyi* remained in the fibrous track.

Evaluation of results

Antitoxin given after wounding was of immense value in preventing the onset of the disease. The untreated contaminated control animals showed a survival rate of 4.6–13 per cent. (within 90 per cent. confidence limits). Those sheep given antitoxin as a schedule commencing 9 hr after contamination showed a survival rate of 94–98.5 per cent. There was, however, more chance of gas gangrene if the administration of the antitoxin was delayed for more than 9 hr. The experimental group given antitoxin after 21 hr could not be given the whole course of injections since all the sheep had died within 48 hr of wounding.

A probit analysis indicated that the likely survival rate when antitoxin was given after a 9-hr delay was 91–99.5 per cent. within 90 per cent. confidence limits and this agrees with the observed values. The survival rate after a 12-hr delay was 82–97.7 per cent. and after a 21-hr delay 15–90 per cent. The confidence band in this area was very wide because of the small number of animals used and thus accurate survival rates could not be predicted. Calculations based on these data indicate that antiserum must be given within 9 hr after challenge to give a 90 per cent. probability of survival.

DISCUSSION

In the animals that were given antitoxin, the predictable pyogenic abscess formed and there was ample evidence that the infecting spores had germinated and multiplied in the wound. There was no sign of the gelatinous oedema that is so characteristic of gas gangrene caused by this organism. Even in those few animals that died of the disease when the antitoxin was withheld for up to 15 hr gas-gangrene myonecrosis was minimal although there were pleural complications which no doubt contributed towards death. Antitoxin, therefore, prevented the extension of the environment necessary for the

anaerobic multiplication of the organisms provided that it was given in time. Delay in administration of antitoxin allowed the fatal process to develop.

Previous attempts to determine the part played by antitoxin in gas-gangrene prophylaxis and therapy following war injuries have been associated with surgical or antibiotic treatment or both, except in the work described by Owen-Smith and Matheson (1968). With the experimental sheep model that we have used, these authors showed that penicillin alone was completely effective in preventing gas gangrene if given within 9 hr of wounding and challenge. By delaying the treatment beyond this time, prophylactic effectiveness decreased and delays of 24 hr or more rendered treatment practically valueless. Owen-Smith (1968) further showed, with a small group of animals, that antitoxin given 9 hr after wounding and 12-hourly thereafter prevented the onset of gas gangrene, although there was evidence of multiplication of the challenge organism.

The results of the present study confirm these observations and show with a large group of animals that antitoxin by itself is effective in controlling the disease in the experimental sheep model if given within 9 hr after wounding. The results that we obtained with prophylactic antitoxin in other groups of sheep show that the relationship is remarkably similar to the results obtained by Owen-Smith with penicillin. One might have expected some difference in the effect of the two forms of treatment, since on the one hand toxic products of growth are being neutralised and on the other the organism itself is being attacked. It is clearly important that the disease should be terminated before it becomes fulminating and in this respect the prophylactic value of antitoxin is an indication of the large part played by toxin in the spread of the disease. The results obtained in the present study and the observations of Owen-Smith indicate that neither antitoxin nor antibiotic used singly, is therapeutically useful in the established case. Once gas gangrene has a hold it rapidly enters an inexorably fulminating stage that ends in death. Accordingly, it would be interesting to test MacLennan's opinion that the prophylactic use of antibiotic agents and antitoxin in combination is of more value than the use of either singly. This should certainly be considered in any further study.

There is no doubt that antitoxin by itself can play an important part in prophylaxis against gas gangrene caused by *Cl. novyi*. However, its efficacy depends upon its being given as soon after wounding as possible and preferably within 9 hr.

SUMMARY

Sheep that were wounded in the thigh muscle by a high-velocity bullet and simultaneously infected there with spores of *Clostridium novyi* type A were used to evaluate the prophylactic effectiveness of gas-gangrene antitoxin. Control sheep that had not received antitoxin almost all died of gas gangrene within 48 hr after wounding (survival rate 4.6–13 per cent. within 90 per cent. confidence limits). The survival rate increased when antitoxin was given prophylactically.

The effectiveness of antitoxin in preventing gas gangrene was found to

depend to a large extent on the interval of time between the challenge and administration of antitoxin. If this was 9 hr or less, antitoxin was almost completely effective (survival rate 94–98·5 per cent.). The survival rate decreased as the interval lengthened; when it was 18–21 hr or more, the rate was similar to that observed with challenged controls.

It is concluded that gas-gangrene antitoxin is likely to be of value in the prevention of post-traumatic gas gangrene caused by *Cl. novyi*, provided that it is given as soon as possible and preferably within 9 hr after wounding.

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