

**Prophylaxis against tetanus in  
non-immune patients with wounds: the role of antibiotics  
and of human antitetanus globulin**

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SUMMARY

The potential value of oral erythromycin for antitetanus prophylaxis in non-immune patients with open wounds was assessed. Serum obtained by venepuncture from healthy persons 2 h after an oral dose of an erythromycin preparation was used as a culture medium rendered anaerobic by addition of cooked meat. Strains of *Clostridium tetani* inoculated into these sera failed to multiply when the donor had taken 500 mg of erythromycin estolate before a meal; other erythromycin preparations and the estolate at a dosage of 250 mg were ineffective or inconsistent in their inhibition of the growth of *Cl. tetani*.

Human antitetanus globulin (ATG) was given to 12 patients, 9 with severe injuries and 3 with extensive burns, all of whom were judged, from their history, to be non-immune (or with expired immunity); all except one had received large intravenous infusions of blood and/or other fluids. Serum antitoxin assays by a mouse protection technique on days 0, 1-2, 3-5, 6-10 and 14+ showed no detectable antitoxin (< 0.01) unit/ml in the initial (pre-ATG) sample from three patients with severe injuries and in one with extensive burns. All the patients in the severely injured group showed an early appearance or increase in tetanus antitoxin to protective titres. Two of the three severely burned patients showed, respectively, a delayed appearance or an increase in antitoxin; the other burned patients showed a reduction from the initial pre-ATG titre, followed by a return to that titre after day 5.

INTRODUCTION

The use of antitetanus horse serum (ATS) for prophylaxis of patients with open wounds went out of favour and was largely abandoned in this country some years ago because the risk of anaphylaxis due to the ATS might be greater than the risk of tetanus, and also because horse antiserum is likely to become ineffective in persons who have received previous injections of horse serum and developed antibodies to its globulins (Wright, 1958; Rubbo, 1966; Sharrard, 1966; Ungar, 1967). A reinforcing ('booster') dose of toxoid is universally recognized to be the correct prophylaxis for a patient known to be actively immune - and even a booster dose is considered to be unnecessary if the course of immunization or a previous booster

dose was given within the previous year. For patients with contaminated wounds who are not known to be actively immune, however, it is necessary to provide immediately effective prophylaxis, and the alternatives available are systemic chemoprophylaxis with an antibiotic active against *Clostridium tetani*, or (preferably) passive immunization with human antitetanus globulin (ATG). Animal studies have shown the potential value of systemic erythromycin, tetracycline and penicillin in prophylaxis against challenge by injections of *Cl. tetani* (Taylor & Novak, 1951; Anwar & Turner, 1956; McDonald, Chaikof & Truant, 1960). Injection of human antitetanus globulin into human volunteers or patients has been shown to give protective amounts of antitoxin in the blood, without the rapid elimination of antibody which is likely and the risk of anaphylaxis which is unavoidable (even with test doses) when animal sera are used (Rubbo & Suri, 1962; Suri & Rubbo, 1961; Ungar, 1967).

In this study we have examined two questions: (1) Is the growth of *Cl. tetani* inhibited by the blood of persons who have been given a standard dose of an antibiotic active against the organism and shown to prevent tetanus in animals? (2) Does a recommended dose of ATG give protective blood concentrations of tetanus antitoxin in patients with severe injuries or burns who have received large infusions of blood, plasma or other intravenous fluids?

#### PROPHYLAXIS BY ANTIBIOTICS

Benzyl penicillin is liable to be inactivated by the penicillinase from *Staphylococcus aureus* and other hospital-acquired bacteria colonizing open wounds; it is therefore not an appropriate antibiotic for prophylaxis against *Cl. tetani*, in spite of its good activity against the organism. The tetracyclines which have broad spectrum activity are also inappropriate for prophylaxis against an organism adequately covered by a narrow-range antibiotic, like erythromycin or a penicillinase-stable penicillin (e.g. cloxacillin or flucloxacillin). We have therefore restricted our studies to antibiotics in these groups; in this paper we report our findings with erythromycin.

#### *Materials and methods*

Healthy volunteers were given single oral doses (250 mg and 500 mg) of erythromycin, erythromycin stearate, erythromycin estolate and a paediatric preparation ('Erythroped Forte', Abbott). In separate experiments volunteers took the dose of erythromycin 1 h before and 1 h after the first meal of the day. Venous blood (15.0 ml) was withdrawn 2 h after the volunteer had taken the dose of erythromycin, when it was expected that the peak concentration of erythromycin might be present. The blood was allowed to clot; serum was separated and transferred to a 3 × ½ in. tube containing minced sterilized cooked meat to a depth of about 1 cm, as in the preparation of cooked meat broth for anaerobic culture. Preliminary tests had shown that *Cl. tetani* multiplied well in this 'cooked meat serum' prepared from blood of persons who had not been given an antibiotic.

One drop of a 1/1000 dilution of a cooked meat broth culture of each of two strains of *Cl. tetani* was inoculated into each of the tubes containing cooked meat

Table 1. Growth of *Cl. tetani* in serum (with cooked meat) from volunteers given dose of erythromycin 2 h before the sample was taken

Preparation	Dosage (mg)	Numbers of tests showing growth of			
		<i>Cl. tetani</i> strain 1 (M.I.C. 0.03 µg/ml)		<i>Cl. tetani</i> strain 2 (M.I.C. 0.25 µg/ml)	
		Dose before meal	Dose after meal	Dose before meal	Dose after meal
Erythromycin tablets B.P.	250	6/6	4/4	6/6	4/4
	500	7/7	4/4	6/6	5/5
Erythromycin stearate tablets ('Erythrocin', Abbott)	250	2/5	4/4	7/7	5/5
	500	2/7	6/6	2/7	4/4
Erythromycin estolate tablets ('Ilosone', Lilly)	250	3/9	4/4	4/9	5/5
	500	0/9	0/6	0/9	0/6
Paediatric ('Erythroped Forte', Abbott)	500	0/4	4/4	4/4	4/4

with serum from volunteers who had been given a single dose of an oral erythromycin preparation; the antibiotic had been given either 1 h before or 1 h after a meal. One of the strains of *Cl. tetani* was at the upper end and one at the lower end of the range of sensitivity shown in a study on 47 strains of the organism (Lowbury, 1971). After 4 days' incubation at 37 °C the tubes were subcultured to horse blood agar plates made with 4% New Zealand agar to prevent swarming. The plates were examined, after 3 days anaerobic incubation at 37 °C, for growth characteristic of *Cl. tetani*; when present, the organisms were identified by morphological examination and characteristic growth on an antitoxin-controlled blood agar spot plate (Lowbury & Lilly, 1958).

Assays of erythromycin in the volunteers' serum 2 h after they had taken the dose of erythromycin were made by inoculation of doubling dilutions of the serum in nutrient broth with a one drop of a 1/250 dilution of an overnight broth culture of *Staph. aureus* (Oxford strain), and making a parallel test with a known range of dilutions of erythromycin. To test the persistence of erythromycin in the volunteers' serum when cooked meat was present, assays were made on seven consecutive days. In a parallel experiment, tubes containing cooked meat and serum from persons not receiving an antibiotic were 'laced' with known amounts of erythromycin base, stearate and estolate, 5 µg ml, and the concentration of the antibiotic was assayed on seven consecutive days.

### Results

Table 1 shows the proportion of tests in which serum from a volunteer who had taken erythromycin two hours previously allowed growth of *Cl. tetani*. None of the tests in which the erythromycin base was taken showed inhibition of the growth of *Cl. tetani*. With the stearate there was inhibition of both strains in most of the tests when 500 mg of the antibiotic were taken on an empty stomach; with the

Table 2. Assay of erythromycin in volunteers' serum 2 h after a dose

Preparation	Dose (mg)	Mean titre of erythromycin $\mu\text{g/ml}$		Number of observations
		Taken before meal	Taken after meal	
Erythromycin tablets	250	0.25	< 0.06	5
B.P.	500	0.25	< 0.06	5
Erythromycin stearate tablets	250 500	0.5 0.75	< 0.06 < 0.06	4 4
Erythromycin estolate tablets	250 500	1.0 1.5	1.0 1.75	5 5
Paediatric	500	0.6	< 0.06	2

250 mg dose this effect was observed when the more sensitive strain of *Cl. tetani* was inoculated into the serum. Both strains of *Cl. tetani* were inhibited in all the tests by the serum of volunteers who had taken 500 mg of the estolate either before or after a meal; 250 mg of the estolate was less effective, and the serum of volunteers who had taken this dose did not inhibit either strain in any of the tests when antibiotic was taken after a meal. Serum from volunteers who took the paediatric preparation inhibited only the more sensitive strain and only when the antibiotic was taken before a meal, though this happened in all four tests.

Table 2 shows the mean titres of erythromycin in the serum of volunteers who had received a dose of the antibiotic 2 h previously. We failed to detect erythromycin in the serum when the base or the stearate had been given after food, possibly because 2 h was an under-estimate of the time required to reach the peak serum concentration. Estolate was the only preparation which gave detectable serum concentrations when taken after a meal. The serum concentrations after taking erythromycin tablets B.P. before a meal were above the M.I.C. of the more sensitive strain of *Cl. tetani*, and yet the organism grew in cooked meat serum containing this concentration of erythromycin.

The activity of erythromycin in cooked meat serum at 37 °C did not decline for 3 or 4 days, but then fell progressively, and was not detectable by the seventh day.

#### PROPHYLAXIS BY HUMAN ANTITETANUS GLOBULIN (ATG)

Rubbo & Suri (1962) showed that protective titres of antitoxin could be obtained for at least 14 days when 400 units of human antitetanus globulin, prepared from the serum of actively immunized persons, were injected into non-immune human subjects. In the United States, Canada, Australia and some European countries human ATG has become adopted in place of ATS as the standard prophylactic for non-immune injured patients, and the same change has recently taken place, with the marketing of a form of ATG ('Humotet', Wellcome), in Great Britain.

Patients with severe injuries or burns are given large intravenous infusions of blood, plasma or plasma substitutes and it might be expected that these would interfere with the prophylactic effect of ATG. The massive exudation from burn

wounds could lead to the loss of injected antitoxins and this might also be diluted by infused fluid. In the following study we have examined this hypothesis.

#### *Materials and Methods*

##### (1) Selection of patients for prophylaxis by ATG

Patients with open wounds admitted to the Major Injuries Unit or with severe burns (of 15% or more of the body surface) admitted to the Burns Unit of the Accident Hospital were given 250 units of ATG ('Humotet'), by intramuscular injection, if the patient was not known to be actively immune to tetanus by the accepted criteria (Lowbury, Ayliffe, Geddes & Williams, 1975).

On the day of admission (day 0), before the first dose of ATG, and again on days 1, 4, 7 and (if possible) 14, 4–5 ml of venous blood were taken for assay of tetanus antitoxin in the serum. The first dose of tetanus toxoid for active immunization of these patients was not given until after the last sample of venous blood was taken for antitoxin assay.

##### (2) Assay of antitoxin in serum

A standard dose (L+/500) of tetanus toxin was mixed with a range of dilutions of serum from patients who had received ATG; the mixtures were injected into the lumbar region of mice near the base of the spine; one mouse was used for each dilution. The titre of antitoxin in the serum was estimated from the highest dilutions which protected the mice, as shown by their survival after 3 days.

The toxin, kindly supplied by Wellcome Research Laboratories, was diluted in borate buffer solution to give L+/250 per ml. Doubling dilutions of the patient's serum from 1/2.5 to 1/100000 were made in borate buffer. 1 ml amounts of the toxin dilution (L+/250) were added to tubes containing 0.4 ml of the serum dilutions. The mixtures were allowed to stand at room temperature in the dark for 1 h; 0.7 ml of each mixture was then injected into a mouse, as described. The mice were examined daily, and records were kept of the development of paralysis of the hind legs and death. The titration was carried out in two stages:

(1) Assay for titres of 0.01 to 0.5 units antitoxin per ml serum, as shown by end-points in the range from neat to a 1/50 dilution of serum, and

(2) If the mice were protected in the first range, assay for titres from 1 to 100 units of antitoxin per ml serum, as shown by end-points in the range from 1/100 to 1/10000 dilutions of serum.

#### *Results*

Table 3 shows the serum antitoxin titres in 9 patients with major injuries and 4 patients with extensive burns who were given 250 units of ATG. Only four of these (one with extensive burns) showed no detectable antitoxin before the ATG was given. In the Major Injuries Unit, patients who were non-immune on admission showed the appearance of antitoxin, and those who were found on admission to have antitoxin showed an increase in antitoxin titre on days 1–2 (or on days 3–5 if no earlier sample was obtained). In the Burns Unit, by contrast, two patients (K and L) showed a fall in antitoxin titre and one, with no initial antitoxin,

Table 3. *Titres of tetanus antitoxin in patients with severe injuries and burns given 250 units of ATG ('Humotet')*

Clinical	Serial number	Tetanus antitoxin, units per ml, in serum on day (after injection)				
		0	1-2	3-5	6-10	14+
Severe injuries	AA	2.5	5.0	—	2.5	5.0
	B	0.01	0.5	—	0.25	0.1
	C	0.25	1.0	0.5	0.5	0.5
	D	< 0.01	—	0.025	0.01	0.01
	E	< 0.01	0.025	—	0.025	—
	F	< 0.01	1.0	1.0	1.0	—
	G	2.5	5.0	5.0	—	5.0
	H	10.0	—	10.0	25.0	—
	I	5.0	10.0	5.0	—	—
Severe burns (41%)* (28%) (15%) (37%)	J	< 0.01	< 0.025	< 0.01	0.25	0.25
	K	0.1	0.05	0.5	0.25	0.25
	L	0.05	0.025	0.025	0.05	0.05
	M†	0.1	—	0.25	5.0	10.0

\* Estimated area of burns.

† Patient given toxoid booster on admission.

showed no detectable antitoxin on days 1-5; patients J and K showed, respectively, the appearance of antitoxin and the increase in antitoxin titre after day 5. Patient L showed no increase in antitoxin titre even on day 14. Patient M showed an increase in antitoxin titre on days 3-5, which rapidly mounted to high titres by days 6-10 and day 14. On checking the notes of this case, we found that the patient had been given tetanus toxoid and not ATG. We include the results in Table 3, because of the good immune response shown in the patient with severe burns.

#### *Volumes of fluid infused into patients given ATG*

All except one of the patients received substantial infusions. Patients with burns were given larger volumes of clear fluid (human plasma protein fraction or dextran) than of blood; patient J received over 9 litres of clear fluids. They were also given large volumes of fluid by mouth. Most of the patients with severe injuries other than burns were given large volumes of blood (e.g., over 6 litres in patient F), and most were also given large infusions of clear fluid.

#### *Proportion of patients 'immune' on admission to hospital*

A wall chart in use at the Birmingham Accident Hospital (Lowbury, Ayliffe, Geddes & Williams, 1975) divides patients into four categories as regards the type of anti-tetanus prophylaxis required. A survey on 719 consecutive patients attending the Casualty Department during February and March 1976 revealed that 342 required no specific prophylaxis (Category A), 300 were considered to be actively immune (Category B), requiring a booster dose but no passive protection by antiserum or antibiotic, and 77 were not known to be actively immune (Cate-

gories C and D); of these 66 were considered suitable for chemoprophylaxis as an alternative to ATG if the wound was not severe.

The patients included in the study of ATG described above were all selected from those in Categories C and D (non-immune), but the majority of these were shown to have pre-immunization titres of antitoxin which were presumably due to active immunization.

#### DISCUSSION

In 1966 Rubbo pointed out that the use of human ATG was gaining widespread acceptance in the United States, Canada and Australia, and expressed surprise that in the UK human ATG was reserved for 'the rare case in which nothing else would be suitable'; 'so long as these views prevail', he added 'one can understand the popularity of antibiotic prophylaxis in the U.K.' In 1974 the Wellcome Foundation made the ATG preparation 'Humotet' available in this country, and its use in place of animal ATS was generally adopted. Because of the cost of 'Humotet', however, there seemed to be a role for prophylaxis by an antibiotic (especially erythromycin) in non-immune patients with smaller contaminated wounds and in whom there was little delay in starting treatment.

Our study on prophylaxis by erythromycin products showed that estolate was the only oral preparation which always gave blood concentrations capable of inhibiting the multiplication of *Cl. tetani* 2 h after a dose of the antibiotic was taken; an adult dose of 500 mg erythromycin estolate gave blood concentrations inhibitory to the less sensitive as well as the more sensitive strain of *Cl. tetani*, and might be expected to have prophylactic value. Because hepato-toxic effects may occur on administration of the estolate for more than 10 days, the course should not be longer than a week, and there is some doubt whether an antibiotic with such potential toxicity should be used for prophylaxis against a remote hazard. The alternatives are penicillin, which is likely to be ineffective because of inactivation by penicillinase from other bacteria in the wound, and the penicillinase-stable penicillins, which are likely to cause the selective emergence of a high proportion of 'methicillin-resistant' *Staph. aureus* if used prophylactically (Ayliffe Green, Livingston & Lowbury, 1977), and which may prove to be no more effective than erythromycin, though about as expensive as ATG.

The evidence of potential effectiveness of ATG from blood titres of antitoxin suggests that severely injured patients should receive good protection from a dose of 250 units ATG, but severely burned patients may not receive such protection until several days after the injection of ATG; the appearance or increase in concentration of antitoxin at that stage, however, suggests that not all of the injected ATG has been lost. After extensive burns several factors may combine to depress the concentration of antitoxin in the circulation. There is a loss of immunoglobulin from the body in exudate; the average daily loss of exudate in our three cases would be about 1½ litres, containing about 45 gms or 1/5 of the circulating plasma proteins. The loss in a burn covering 80% of the body surface might be twice this amount. The ATG injected into these cases was diluted after absorption with globulin-free Human Plasma Protein Fraction and Dextran, which were given to

restore the plasma volume. In extensive burns the extracellular fluid space may also be expanded with exudate by as much as 50%, and this would increase the dilution of absorbed ATG. The antitoxin which is sequestered in the burn oedema may not get back into the circulation till the oedema is absorbed about a week later.

These features of extensive burns are in contrast to many severe non-burn injuries. In these there may be early loss and replacement of blood, but the operation is usually within 6 or 8 h of injury and loss of blood and ATG then cease. Oedema from these injuries is often less than that which occurs even in a 30% burn.

The imperfections both of antibiotic prophylaxis and of passive immunization shown in these studies emphasize the need to ensure that as many healthy people as possible have the benefit of active immunization. It was encouraging to find that a majority of the patients attending the Casualty Department in this Hospital were considered or shown to be actively immune on admission.

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#### REFERENCES

- ANWAR, A. A. & TURNER, T. B. (1956). Antibiotics in experimental tetanus: *in vitro* and *in vivo* studies. *Bulletin of Johns Hopkins Hospital* **98**, 85-101.
- AYLIFFE, G. A. J., GREEN, W., LIVINGSTON, R. & LOWBURY, E. J. L. (1977). Antibiotic-resistant *Staphylococcus aureus* in dermatology and burn wards. *Journal of Clinical Pathology* **30**, 40-4.
- LOWBURY, E. J. L. (1971). *Folia Traumatologica Geigy: Tetanus*. Basle: Ciba-Geigy.
- LOWBURY, E. J. L., AYLIFFE, G. A. J., GEDDES, A. M. & WILLIAMS, J. D. (ed.) (1975). *Control of Hospital Infection: A Practical Handbook*, p. 216. London: Chapman and Hall.
- LOWBURY, E. J. L. & LILLY, H. A. (1958). Contamination of operating theatre air with *Clostridium tetani*. *British Medical Journal* **ii**, 1334-6.
- MCDONALD, R. T., CHAIKOF, L. & TRUANT, J. P. (1960). An analysis of prophylactic measures in experimental tetanus. *Surgery, Gynecology and Obstetrics* **110**, 702-6.
- RUBBO, S. D. (1966). New approaches to tetanus prophylaxis. *Lancet* **ii**, 449-53.
- RUBBO, S. D. & SURI, J. C. (1962). Passive immunization against tetanus. *British Medical Journal* **ii**, 79-81.
- SHARRARD, W. J. W. (1966). Prevention of tetanus. *British Medical Journal* **i**, 105.
- SURI, J. C. & RUBBO, S. D. (1961). Immunization against tetanus. *Journal of Hygiene* **59**, 29-48.
- TAYLOR, W. I. & NOVAK, M. V. (1951). Prophylaxis of tetanus with penicillin-procaine. *Annals of Surgery* **133**, 44-9.
- UNGAR, J. (1967). Heterologous antitoxin and homologous immune globulin in tetanus prophylaxis. In *Principles on Tetanus* (ed. L. Eckman), pp. 409-416. Bern: Huber.
- WRIGHT, G. PAYLING (1958). The use of ATS in the treatment and prophylaxis of tetanus. *Proceedings of the Royal Society of Medicine* **51**, 997-1000.