

## Comparative trial of three heterologous anti-tetanus sera

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### SUMMARY

The three heterologous anti-sera currently provided for tetanus prophylaxis have been compared with reference to the production of untoward reactions in 498 patients, and to the blood antitoxin concentrations produced in 76 patients. Equine serum, although giving rise to more reactions, was the only effective agent in terms of the levels and duration of serum antitoxin concentration produced. The local response to a test dose of any of the three sera is not a reliable guide to immediate or late general reactions.

### INTRODUCTION

There is universal agreement that the essential factor in the prevention of tetanus following injury is prompt and adequate surgical attention. By contrast, conflicting views have been expressed in recent years regarding the place of equine anti-tetanus serum as an additional, specific prophylactic agent. For low-incidence areas, such as Britain and the United States of America, Rubbo (1966) based a strong case against the continued use of equine antiserum on the known dangers of morbidity and mortality that may follow its administration. In addition, hypersensitivity to equine serum results in accelerated elimination and reduced efficacy of the serum in some patients. Alternatives to the use of equine antiserum for the prophylaxis of tetanus include the administration of prophylactic antibiotics and the use of antisera developed in other animals or in man.

Experimental work with animals has cast doubts on the degree of protection afforded by penicillin to patients with wounds that have received delayed or inadequate attention (Smith, 1964). Important considerations here include the possibility of coexistent pyogenic infection with organisms that produce penicillinase, the persistence of resistant tetanus spores, and inadequate access of antibiotics to areas of ischaemic tissue. Misgivings have understandably been expressed concerning the prophylactic administration of antibiotics for this purpose, as in other situations such use is considered inadvisable. Moreover, there is no general agreement on either the choice of antibiotic or what constitutes a fully protective dosage schedule. Most authorities accept that the alternative or additional protection of antiserum should be given in some cases. As a result of these different considerations various codes of practice now exist for the prophylaxis of tetanus and a bewildering series of recommendations confronts the casualty officer. Despite

guidance given by the British Ministry of Health Advisory Group (1964) on this subject, we feel that the situation requires further clarification particularly with regard to the use of prophylactic antiserum. At present three heterologous anti-tetanus sera ('ATS') of equine, bovine and ovine origin, are produced in this country whereas a supply of homologous anti-tetanus globulin (ATG of human origin), which would resolve many of the problems, is not yet generally available in Britain. It is not our wish to enter here into the controversy regarding the relative place of homologous and heterologous sera. The purpose of the present paper is to present our experience of the three heterologous sera (equine, bovine and ovine ATS) that are currently available and officially recommended for clinical use. Our observations concern untoward reactions produced, and the serum antitoxin titres achieved and maintained in the patient.

#### MATERIAL AND METHODS

Pepsin refined bovine, equine and ovine anti-tetanus serum was supplied in ampoules by the Wellcome Research Laboratories. Each ampoule contained a single dose of 1500 i.u. antitoxin labelled A, B or C respectively, but the identifying code was unknown to any member of the clinical team. Ampoules containing the vehicle without any immune globulin were also available as controls. Patients were considered for the trial consecutively as they presented themselves at the Accident and Emergency Department during the period of January to June 1968. All those with a definite history of previous active immunization were treated with a booster dose of tetanus toxoid, and all those under 21 years of age were excluded from the trial. All other patients for whom specific tetanus prophylaxis was necessary were considered eligible. The form and purpose of the trial were explained to each individual and only those who consented were included. A careful history was taken from each subject regarding previous immunizations and allergies and this information was later checked as far as possible with the Public Health Authorities and with the general practitioner concerned. The basis of each patient's treatment was surgical toilet of the wound and administration of ATS, but a definite history of allergy was regarded as a contra-indication to serum prophylaxis. Every patient received an intramuscular injection of adsorbed tetanus toxoid. Antibiotics were not prescribed as prophylactic agents against tetanus, but were used if considered clinically desirable when pyogenic infection was diagnosed.

The antiserum was selected by a predetermined random pattern on a weekly rotation. Each patient was given a subcutaneous test dose consisting of 0.1 ml. of the 'serum of the week' (A, B or C). Simultaneously a control injection of the vehicle was given at a corresponding position on the opposite forearm. The patient was observed over a period of 30 min. for the development of any local or general reaction, and the findings were recorded. A positive local reaction was arbitrarily defined as a wheal measuring 1 cm. or more in diameter. Any local reaction less than this was recorded but the full dose of the serum was administered by intramuscular injection. If the local response was a wheal greater than 1 cm. in diameter, test doses of the other two sera were given, and the patient received the

full dose of the serum to which the local reaction was least marked. As the trial progressed it was confirmed that a local reaction consisting of a wheal greater than 1 cm. was not evidently associated with any increased risk of an immediate general reaction and in many cases in the later part of the trial the full dose of the serum was given despite such local effect. The position of each injection was standardized and marked with indelible ink, and the patient was reviewed on his return for wound treatment. Any reaction or history of reactions was recorded and return visits arranged for the patients concerned. The general practitioners in the region, who had previously been consulted through the local medical committee, were asked to report any possible reactions to ATS that developed in their patients included in the trial. A suitable form was supplied for the return of this information. The patients themselves were contacted by postal survey at the end of the trial to obtain information about any late reactions which had not been observed by ourselves or reported by the family doctor.

Samples of venous blood were taken from approximately every sixth patient in the trial for assay of antitoxic activity before the first injection and subsequently on the 5th, 10th, 14th and 21st days thereafter, or as close to these days as could be arranged. Defaulters were visited at their homes to complete the taking of blood samples. The serum was separated immediately and dispatched to the Wellcome Research Laboratories where the antitoxin content was assayed by *in vivo* titration using the mouse subcutaneous method (Glenny & Stevens, 1938). At the end of the trial, the potency of the three antisera was rechecked, and no significant deterioration had occurred.

## RESULTS

The trial included 498 patients on whom clinical data were completed, and antitoxin titres were followed in 76 of these patients. The data were analysed by the Wellcome Foundation on an ICL computer; 205 patients received serum A (bovine), 148 serum B (equine), and 145 serum C (ovine).

### REACTIONS ATTRIBUTABLE TO SERUM

The reactions produced by the test doses were compared with those produced by the injection of vehicle alone. It was found that a zone of erythema up to 1 cm. in diameter was occasionally produced by the vehicle and it was, therefore, concluded that such a local reaction was not necessarily attributable to the protein in the injection and could be ignored. Local reaction to the test dose was recorded by measuring the diameter of the wheal on three occasions during the 30 min. following injection. Because of the work load in the Accident Department precise times of recording could not be achieved and three time brackets of 5 min. were therefore used (Table 1). Reactions greater than 1 cm. were more common with equine serum than with either bovine or ovine (7.4, 4 and 2.8 % respectively) but this difference is not statistically significant.

There was no immediate general reaction to a test dose of any serum or to a full dose of bovine or ovine serum; a full dose of equine serum may have been

responsible for an immediate, general reaction in one patient who felt faint and nauseated, became hypotensive and was seen to hyperventilate. This reaction was brief and self limited. The patient had not shown any reaction to the test dose.

Table 1. *Incidence of local wheals after subcutaneous injection of 0.1 ml. of antiserum*

Serum	No. of cases	No. showing induration of $\geq 1$ cm. in diameter at		
		15-20 min.	20-25 min.	25-30 min.
Bovine	205	4	6	8 (4)
Equine	148	13	12	11 (7.4)
Ovine	145	2	4	4 (2.8)

The figures in parentheses are the percentage positive after 25-30 min.

Table 2. *Incidence of late reactions (local and general) occurring after a full dose of the three antisera*

Serum	No. of patients	No. of patients showing reactions			
		Total	Local	General	Both
Bovine	205	25 (12)	18	7 (3)	0
Equine	148	42 (28)	26	16 (11)	2
Ovine	145	17 (12)	8	9 (6)	2
All sera	498	84 (17)	52	32 (6)	4

The figures in parentheses are percentages.

Table 3. *Details of late general reactions occurring after a full dose of the three anti-sera*

Serum	Adenitis	Rash	Arthritis	Other	Days off work
Bovine (7 cases)	3	4	1	—	2
Equine (16 cases)	4	9	3	4 (giddiness and headaches)	11 (1 case off and 7 days with skin rash)
Ovine (9 cases)	2	5	1	2 (sickness and headaches)	$\frac{1}{2}$

Of the delayed reactions, local induration in the buttock was by far the commonest, and such local reactions to the full dose were significantly more common after equine serum (Table 2).

Delayed general reactions, or symptoms that could be attributed to this cause were observed in 7 out of 205 patients given bovine serum, 16 patients out of 148 given equine serum and in 9 patients out of 145 given ovine serum (Table 2). The increased incidence after equine serum is again significant. Of these 32 patients

only 2 had shown a local reaction to the test dose. More detailed analysis of the late general reactions is summarized in Table 3. Equine serum was responsible for the loss of 11 working days whereas bovine and ovine serum caused the loss of 2 days and one half day respectively. (The morbidity for equine serum was considerably increased by one patient who was away from work for 7 days on account of a skin reaction.)

### Efficacy

A serum antitoxin concentration of more than 0.01 units per ml. was regarded as the minimum protective concentration (King, Kaiser, Lempke & Ruster, 1963; McComb, 1964; White *et al.* 1969). The necessary duration of such a concentration will depend on the wound concerned but a period of 10–14 days would give protection in the majority of cases.

Table 4. Percentage of patients with predicted serum anti-toxin titres greater than 0.01 units per ml. at various times after the injection of 1500 units of anti-toxin

Serum	Day 1	Day 2	Day 7	Day 10	Day 14
Bovine (22 patients)	68	64	55	22.7	14
Equine (16 patients)	100	100	100	100	88
Ovine (13 patients)	62	62	23	8	15

Analysis of the data obtained was complicated by two factors. First, our decision to give all patients in the trial simultaneous active immunization. When the passive protective potency of the serum came to be assessed, of the 76 patients in whom adequate serial assays were available 25 were excluded because of either high initial serum antitoxin titres or an early secondary immune response to the toxoid injection. Secondly, there was inevitably some variation in the days after injection on which blood samples were taken so that direct comparison of the titres for the three sera would be invalid. Bias due to this factor was removed by utilizing regression equations, calculated by plotting the logarithms of the titres against the elapsed time, to predict responses at a fixed time and then analysing these predicted responses. Table 4 shows the percentage of subjects who would be predicted to have protective antitoxin titres at various times as calculated from the data for the 51 'valid' cases. There is a very obvious and statistically significant difference ( $p = 0.01$ ) between the patients given equine serum and those given the other two sera.

### DISCUSSION

However strongly we may advocate universal active immunity as the proper approach to tetanus prophylaxis this ideal will not be readily achieved and the need for a passive protection of susceptible injured patients will continue to arise. A study of 3000 consecutive patients attending the Accident and Emergency Department in the early spring of 1968 showed that, of 629 patients considered

to require some form of prophylaxis against tetanus, 140 (22%) claimed to have been actively immunized. In Leeds, after years of active campaigning, the proportion of patients attending the Accident Department in 1966 who had been actively immunized was 63.4% (Ellis, 1967). In assessing the relative merits of serum and antibiotics in prophylaxis, the theoretical reasons that would justify preference for serum are set against dangers that attend the normal clinical use of equine anti-serum. These dangers will be markedly reduced if human serum is used but as this may not be widely available in Britain for some years it has been suggested that anti-sera should be prepared in animals other than the horse. Although Trinca & Reid (1967) found that bovine antitoxin produced few untoward reactions, the serum antitoxin concentrations achieved by the injection of bovine anti-serum in his patients were not recorded. In the present study we have confirmed that equine serum carried a higher morbidity than the other two sera though the loss of working days was much less than that found in Sheffield by Cox, Knowelden & Sharrard (1963), being 7 days per 100 patients in the present series as against 24 days per 100 patients in the Sheffield series.

More important, however, are the relatively low blood titres produced by the two sera which are at present officially provided by the Ministry of Health for use when equine serum has proved unsuitable. Whereas equine serum was predicted to give protective titres in 100% of subjects at 10 days, bovine serum gave predicted protective titres in only 23% at 10 days and ovine serum in only 8%. It is difficult to explain this striking difference in efficacy. If any one of the three sera should have been eliminated more rapidly than the others this should have been the equine serum, because of previous exposure of many patients to this antigen. Moreover, accelerated elimination could not explain the findings because, in a small number of patients in whom serum antitoxin titres were assayed during the first 24 hr. there was no evidence of an adequate but earlier titre in the bovine and ovine groups, while the slope of the curves obtained by plotting log titres against elapsed time, and which could be taken to indicate the rate of decline of antitoxin titres, was similar for all three sera. Although the test groups were small it appears doubtful whether bovine and ovine anti-tetanus sera should continue to be regarded as acceptable alternatives to equine serum.

This conclusion is particularly important as there does not yet appear to be official support for increased production of human anti-tetanus globulin in this country. Our present evidence suggests that human ATG should now be at least the main alternative to equine serum and should entirely replace it as soon as is practicable. This view is supported by practice in other developed countries. An alternative solution might lie in means of reducing the antigenicity of horse globulin in man. Although some have claimed that ultracentrifuged preparations of bovine gamma-globulin have reduced antigenicity in mice (Dresser, 1962) and rats (Gery & Waksman, 1967) attempts by one of us to repeat this work with bovine and equine gamma-globulin in rats have so far been unsuccessful.

Although it has been standard teaching for many years that the value of a test dose of serum in predicting dangerous sensitivity lies only in the development of an immediate general reaction to the test dose, we have repeatedly observed

reluctance on the part of doctors to give a full dose of anti-serum to a patient who has shown a local wheal, or even erythema, in response to the test dose. Our experience confirms that the subcutaneous test dose is of no practical value in predicting delayed reactions to the sera (Laurent & Parish, 1962), and this finding applies equally to all three sera. This view has been contested by Binns (1961) who concluded, in a study of 3455 patients, that the local reaction was important in relation to the development of delayed general reactions. However, he did not state in detail the nature of these responses, save that two of them, occurring up to 50 min. after the test dose, were severe anaphylactic reactions. Certainly in our series, none of the delayed reactions was sufficiently severe to warrant the replacement of serum prophylaxis for that particular injury by any other form of therapy, and in only one of these cases was there an immediate reaction to the test dose of induration 1 cm. or more in diameter.

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