CLINICAL TRIAL OF COAGULASE AND ALPHA-HAEMOLYSIN TOXOIDS IN CHRONIC FURUNCULOSIS

BY

K. JOY HARRISON, M.D., D.C.H., D.Obst.R.C.O.G.

Pathological Department, General Infirmary, Salisbury

With the advent of antibiotics it was hoped that a cure for furunculosis had been found which would strike at the cause of the infection and obviate the need for immunization. It soon became apparent that, although the use of an appropriate antibiotic cured the boil present at the moment, the occurrence of further boils was not prevented (Scott and Waterworth, 1958), and current thought is now turning again to active immunization.

The wide-spectrum antigenic stimuli of earlier workers having failed to confer immunity, it seemed reasonable to try the effect of immunization with a single toxin to see whether the neutralization of one noxious factor would break a link in the defensive chain of the staphylococcus and render it vulnerable to the body's attack. Kemkes (1928) pointed out that there appeared to be a direct relationship between the production of alpha-haemolysin and coagulase and the virulence of the staphylococcus ; and work by Ramon (1936), Downie (1937), and Boake (1956) has supported this view. Although the exact roles of coagulase and alpha-haemolysin have not been determined it is certain that they both contribute to the offensive armamentarium of the staphylococcus.

For this reason and because of their availability to me in pure form, these two toxins were chosen for therapeutic trial in toxoid form together with a placebo. The aim of the trial was to test the efficiency of the toxoids in preventing recurrent boils, and they were never regarded as having any part in the treatment of an individual boil. Practitioners were free to give treatment, including antibiotics, for the boils according to their personal preference and as the clinical condition demanded.

Substances Used for Injection

The three substances used in the clinical trial were staphylococcus toxoid *B.P.*, beta-propiolactone coagulase toxoid, and a placebo. The staphylococcus toxoid was made and donated by Glaxo Laboratories Ltd. and packed in phials indistinguishable from the placebo except by code number. The toxoid is a formolized preparation of alpha-haemolysin and contains no coagulase or leucocidin. The placebo, which Glaxo Laboratories also prepared, contained only physiological saline and 0.013% thiomersalate.

Coagulase toxoid was made from coagulase prepared according to the method of Duthie and Haughton (1958), using *Staphylococcus* M_1 and *Staphylococcus* Newman (N.C.T.C. 8178), hereinafter called D_2. These organisms are from phage groups I and III respectively and produce antigenically distinct coagulases. The majority of human staphylococcal infections are caused by organisms from these two groups, and by mixing the two coagulases it was hoped to cover a wider antigenic range than with one only. The purified coagulase was converted to toxoid with 0.2% beta-propiolactone and adsorbed on to aluminium phosphate gel in a ratio of 20 units of toxoid on 1 mg. of adjuvant suspended in 1 ml. of physiological saline. As a preservative 0.013% thiomersalate was added.

Organization and Conduct of the Clinical Trial

Selection of Cases.—Patients, who were referred by local practitioners, were required to have suffered from boils for at least two months, never to have been immunized against boils, and to be over 14 years old. Cases of pustular acne were not included. Patients who were able to attend the hospital for injection were given coagulase toxoid, and those who wanted their general practitioners to continue the injections were allocated alternately to the groups receiving alpha-haemolysin toxoid or placebo.

Dosage.—A test dose of 0.05 ml. of the appropriate material was given at the first attendance. Alpha-haemolysin toxoid was used subcutaneously, and after 0.1 ml. and 0.2 ml. had been given the dose was increased by 0.2 ml. each time until a volume of 1 ml. was reached, when three injections of this amount were given. Placebo was also given according to this schedule. Coagulase toxoid at a strength of 20 units/ml. was injected intramuscularly once or twice weekly. The total dose in each course was 2 ml., usually divided into eight doses of 0.25 ml. Many patients could easily tolerate up to 0.5 ml. in one injection and a number received this amount, thus reducing their total number of injections.

Follow-up.—Patients were asked to return about 10 days after their course of injections was finished for assessment, for a blood sample to be taken, and for explanation of the follow-up letters, which included a questionary to be filled in, detached, and returned. Patients were also given a card on which to note down the approximate duration of any boils, so that these records could be compared with the patient's own assessment of his condition. Patients were told to mark themselves cured only if they had no boils at all since the previous report.

For the assessment of results a scheme of scoring was adopted. From the data obtained from follow-up letters and visits figures were allotted thus: cured, 0 ; improved, 2 ; same or relapse, 3 ; worse, 4. If a patient was having such bad boils after six months that he begged to be given another course of injections his total score was automatically recorded as 15. After a year's follow-up it was therefore possible to judge the cure or severity of boils during this time by the total score, and to ascertain the duration of freedom and the time of relapse by reference to the sequence of figures. Thus a patient with the figures $0+0+3+3=6$, would be seen to have relapsed after four months' freedom from boils. Assessment of the overall progress during the year was made by interpreting a patient's total score as follows. Total score (inclusive): 1–2, much improved ; 3–6, improved ; 7–15, same.

Results

Reactions to Injections

Alpha-haemolysin toxoid did not usually produce much reaction until a dose of 0.4 ml. was exceeded ; 0.6 ml. sometimes produced a raised tender area of erythema up to 4 cm. in diameter which was maximal between the first and third days after injection. The reaction increased slightly with each subsequent injection, and in some patients 1 ml. gave rise to a tender indurated area 6 to 8 cm. in diameter lasting four or five days, with aching and stiffness of the arm. There was considerable individual variation in the reaction, and in no case was the immunization

absolutely painless. Of the 54 cases in this group, 5 (9.3%) had severe, 18 (33.3%) moderate, and 31 (57.4%) slight reactions.

With coagulase toxoid the local reaction was usually slight and there was little or no erythema. The actual injection was painless, but patients often complained of aching in the area, starting about 12 hours after injection and lasting up to 36 hours. The hard lump which formed disappeared gradually in about two weeks. Of 83 patients given coagulase toxoid 49 (58.5%) had slight reactions, 24 (29.5%) moderate reactions, and 10 (12%) severe reactions. Five of the last-mentioned complained of general malaise, headaches, and joint pains after injections on one or two occasions. Of the 40 patients who were given the placebo, none had any visible reaction, but four complained of aching for a few hours at the site of injection.

Bacteriology

Coagulase-positive staphylococci were isolated from the lesions of 143 (84.5%) of the 171 patients treated. The bacteriological findings are analysed in Table I. In the early days of the boil clinic only bacteriophage type 80 was available in the hospital laboratory. Unfortunately, therefore, 32 of the 143 coagulase-positive staphylococci were designated merely "type 80" or "not 80." It will be seen from Table I that the largest number of strains fell in group I.

TABLE I.—*Analysis of Bacteriological Findings in* 171 *Patients Attending the Boil Clinic*

Phage Group, etc.	Patients		Phage Group, etc.	Patients	
	No.	%		No.	%
I	73	42·8	No lysis ..	1	0·5
II	21	12·3	"Not 80" ..	32	18·7
III	10	5·9	Not swabbed	19	11·1
IV ..	2	1·1	No staph. isolated ..	8	4·7
Miscellaneous	5	2·9			

Thirteen patients came to the boil clinic again some months after their first visit, having suffered a recurrence after a period of freedom from boils. It was found that 10 of these were now infected with a staphylococcus of a different phage pattern from the one originally isolated.

Bacterial sensitivity was routinely performed against penicillin, streptomycin, chloramphenicol, tetracycline, and erythromycin. Of 103 strains tested, 54 (53%) were penicillin-sensitive and 50 (48.8%) were sensitive to all five antibiotics, two of these being phage type 80.

Serum Antibody Levels

Of 160 patients with boils whose sera were examined before immunization, 13% had alpha-haemolysin antibodies only, 21% had coagulase antibodies only, 25% had both, and 41% had neither.

Alpha-haemolysin antibodies were assayed by the method of Lack and Wailling (1954). Serum levels before immunization ranged between undetectable amounts and 10 units/ml., the majority being between 0 and 4 units; 89% of the titres were not above the maximum normal of 2 units/ml. (Dolman, 1935).

No initial anticoagulase titre was found in 55% of the 160 patients, 20% had M_1 coagulase antibodies only, 11% had D_2 coagulase antibodies only, and 14% had both types, the titres ranging from nil to 1 in 40. I have found that the majority of normal people (88% of blood donors) have anticoagulase titres of 1 in 8 or less, although titres up to 1 in 64 may rarely occur in the absence of overt staphylococcal infection (Harrison, 1961).

After immunization with alpha-haemolysin toxoid, 40 out of 50 patients (80%) showed a rise in titre, 6 (12%) showed no change, and in 4 (8%) the antibody level fell. The average rise in titre was 4.85 units/ml., and the initial titre was 2 units/ml. or less in all but 2 of the 40 patients; in these two it was 8 units.

After immunization with coagulase toxoid most patients showed a very satisfactory rise in antibody level (see Table II), the average titre being 150. Titration of sera from patients approximately six months after immunization showed that the titre of the majority had fallen to 1 in 40 or below. Two patients still had titres of 80, five of 120, one of 160, and one of 480. The last of these was suffering from continuous severe boils, and it appears that anticoagulase levels fall to 1 in 5 or less by about five months after immunization unless antibody production is stimulated by staphylococcal infection of the same antigenic type.

TABLE II.—*Distribution of Anticoagulase Titres in* 110 *Patients About* 10 *Days After Immunization with Coagulase Toxoid. (Antibody Levels Expressed as Reciprocals)*

Titre	Patients		Titre	Patients	
	No.	%		No.	%
0–10	14	12·6	161–200 ..	—	0
11–20	11	10·0	201–240 ..	8	7·5
21–40	18	16·2	241–320 ..	10	9·1
41–80	13	12·0	321–480 ..	5	4·5
81–120 ..	15	13·5	481–640 ..	9	8·3
121–160 ..	7	6·3			

As the placebo was antigenically inert the serum titrations at the end of the course have the same significance as the initial titrations, and changes in antibody titre reflect only the course of the patient's disease during the injection of the placebo.

Results of One-year Follow-up

The results obtained from follow-up during the year after immunization are shown in Table III. Excluding all patients who were completely free from boils after the injections, the average total score for the year was calculated for each treatment group in the way indicated earlier. The average total score for both the alpha-haemolysin and the coagulase toxoid groups was 7.2 and for the placebo group 6.6. All these results fall in the arbitrary category of "no improvement."

TABLE III.—*Clinical State of* 181 *Patients for One Year Following Immunization*

Type of Injection	No. of Cases	Cured		Much Improved		Improved		Same	
		No.	%	No.	%	No.	%	No.	%
Placebo ..	40	5	12·5	6	15·0	15	37·5	14	35·0
α-Haemolysin toxoid ..	62	19	30·5	2	3·2	8	12·8	33	53·5
Coagulase toxoid ..	79	15	19·0	8	10·0	22	28·0	34	43·0

Despite the impression given by the alpha-haemolysin cure rate of 30.5%, statistical analysis of Table III and other figures not given here reveals that neither of the test substances has any particular advantage over the placebo as a form of treatment for chronic furunculosis. The possibility was considered that a short period of immunity might have been produced which was not apparent in any of the analyses already done. The clinical state of the three treatment groups was therefore compared during the first and second six-months periods after immunization. It was found that the figures for the later period closely paralleled the earlier in all three groups. If an early benefit had worn off, the second six-months period would have

shown a fall in the percentage of patients with no boils and an increase in the number with no improvement. A booster dose or a second course of immunizing agent would then have been indicated.

Discussion

Significance of the Placebo Group

In his study of staphylococcal infections in general practice, Roodyn (1954) found that only 16 (21%) of his 81 cases relapsed, and of that number four patients had between 10 and 18 months' freedom before recurrence. In his later study Roodyn (1960) found that 73% of his patients had a single short attack of boils with spontaneous cure, and another 16% had long remissions. The spontaneous-cure rate reported here is lower than Roodyn's because all the patients in the clinical trial had at least a two-months history of boils and therefore tended to be cases which had already proved refractory to treatment. In view of the occurrence of long remissions it is felt that follow-up should be continued for at least a year, or preferably 18 months, before cure is claimed, and that a control series treated with placebo should be included in the clinical trial of any new treatment for boils.

Reactions to Immunization

Reactions to the injection of alpha-haemolysin are similar to those reported by Murray (1935) and by MacDonald and Taylor (1951), who found that some discomfort was the rule and that its severity was proportional to the dose. Dolman (1935), however, found that the reaction was so slight as to be almost unnoticeable in many of his patients, and that it was greater initially, decreasing after subsequent injections.

Of the 10 (12%) patients who had severe reactions to coagulase toxoid, it is noteworthy that the five who complained of headache and joint pains had fairly severe boils at the time of injection. One of the two patients who developed purpura was among the few in the coagulase group whose injections were given by his own practitioner, and the purpura was not reported until after the conclusion of the course. For this reason no blood investigations were carried out. The other patient's purpura developed after his fourth injection when he was suffering from a severe infection of the foot arising originally from a paronychia of the great toe. He was admitted to hospital on account of this and no more toxoid injections were given. He had a neutrophil polymorph leucocytosis with a total white-cell count of 13,000/c.mm. and a normal platelet count. It is not possible to say definitely whether the purpura was due to the coagulase toxoid or to his concurrent severe infection, but in view of the preceding case it seems possible that the toxoid was responsible.

In Table IV a relationship between the severity of the reaction and the therapeutic response can be seen in patients treated with alpha-haemolysin toxoid. The percentage of those cured, improved, or the same after one year is directly related to the severity of the reaction, and all patients having a severe reaction were completely cured for the follow-up period of one year. No such relationship is apparent in the coagulase group.

TABLE IV.—*Response to Therapy After One Year and the Severity of Reactions in 82 Patients Immunized with Coagulase Toxoid and 53 Patients Immunized with Alpha-haemolysin Toxoid*

Result	Reaction to Alpha-Haemolysin Toxoid						Reaction to Coagulase Toxoid					
	Severe (5)		Moderate (18)		Slight (30)		Severe (10)		Moderate (24)		Slight (48)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cured ..	5	100	10	53	7	23	1	10	3	13·0	13	27·7
Improved ..	—		1	5	5	16	6	60	16	65·2	17	34·2
No change ..	—		7	42	18	61	3	30	5	21·8	18	38·1

Bacteriology

Rountree (1953) and Roodyn (1954) both found group III staphylococci predominant in infections they reported. The predominance of group I in the present study is due to the fact that type 80 comprised 49.5% of all typable strains. The histories of these 53 patients with type 80 infections reveal that 24 of them had a close association with a hospital, being patients, hospital workers, or their near relatives.

In 16 cases of recurrent boils reported by Roodyn (1954) and in his later study also (Roodyn, 1960) the staphylococcus recovered from the initial lesion was identical with that obtained from the subsequent lesion, and he concluded that recurrence was due to failure to eliminate the particular strain harboured by the patient. Gould (1955) produced group III penicillin-resistant staphylococci by serial subculture of penicillin-sensitive staphylococci of groups I and II in increasing concentrations of penicillin. Knight *et al.* (1956) suggested that if this should occur *in vivo* it would account for sudden changes in type found in nasal carriers. This cannot be the explanation for the change in type found in the 10 cases of recurrence under discussion, because none of them had a long course of antibiotic. It appears, therefore, that replacement by a fresh strain is the probable explanation.

Significance of Alpha-haemolysin and Coagulase Antibodies

The findings of Parish *et al.* (1934), Murray (1935), and Dolman (1935) that normal serum anti-alpha-haemolysin levels are the rule in patients with cutaneous staphylococcal infections have been confirmed, and rises in titres of the same order as theirs have been obtained. Murray (1935) was impressed by the rapid clinical improvement in cases which showed a substantial rise in antibody titre and suggested the prognostic value of this. In the present series the cessation of boils appears to have no relation to the serum anti-alpha-haemolysin titre, and I am in agreement with the statement of Dolman (1935) that there is no definite minimum amount of antitoxin above which freedom from staphylococcal infection can be guaranteed.

It is impossible to compare the anticoagulase titres with those of other workers because their methods, materials, and units all differ considerably from those used in this investigation. The levels of M_1 and D_2 anticoagulase in unimmunized patients all fell within the normal range and this appeared at first to parallel the established findings regarding anti-alpha-haemolysin. Very good serum M_1 and D_2 anticoagulase levels were obtained by immunization, but during the conduct of the trial it soon became apparent that the anticoagulase levels as titrated bore no relation to the clinical state of the patient.

Coagulase-neutralization tests were therefore carried out in a number of cases before and after immunization by titrating the patients' sera against coagulase prepared from the staphylococcus isolated from their own boils and against M_1 and D_2 coagulase simultaneously. In some sera the titres obtained were similar to the M_1 or D_2 levels, but in others there was great discrepancy, and a selection of these cases with their clinical course is shown in Table V. From these results it appears that many patients possessed

an anticoagulase antigenically distinct from either M_1 or D_2. It is apparent also that their clinical state does not depend on the serum-anticoagulase level, whether naturally or artificially induced ; for whereas Patients F and G, who had titres of 320, were much improved, Patient C with a titre of 160 showed no improvement whatever. It was therefore demonstrated that the low alpha-haemolysin titres in untreated patients were not, as at first thought, accompanied by similarly low anticoagulase titres, for the titration of patients' sera with coagulase from their own organisms revealed some titres greater than 1 in 320. The situation is further complicated by the knowledge that a number of patients all infected with the same organism, phage type 80, showed no constant pattern of anticoagulase response, and it seems that the antigenicity of coagulase is almost strain-specific, so that the types of coagulase administered would be of benefit only to those few patients infected with the same antigenic types of staphylococcus.

TABLE V.—*Anticoagulase Titres on a Post-immunization Serum Sample from Seven Patients, Showing Discrepancy Between the Titres of the Different Antigenic Types, and Their Clinical Course. (Follow-up Results Expressed as Serial Figures According to the Scheme Detailed in Text)*

Patient	M_1 Anti-Coagulase Titre	D_2 Anti-Coagulase Titre	Own Staph. Coagulase Titre	Follow-up Result During Year	Clinical State During Year
A ..	0	0	7·5	2233	Not improved
B ..	40	20	0	0303	Improved
C ..	20	15	160	3333	Not improved
D ..	240	480	40	3333	Not improved
E ..	60	120	0	3322	Not improved
F ..	10	7·5	320	1003	Improved
G ..	40	80	320	0000	Cured

Conclusion

A high spontaneous-cure rate for furunculosis has been revealed, but neither alpha-haemolysin nor coagulase toxoids have proved better preventives than the placebo. There seems to be an essential similarity between the results of alpha-haemolysin and coagulase immunization in that both appear to give antitoxic rather than antibacterial protection. They both prolong the survival time in animals but do not give complete protection against staphylococcal challenge and afford no benefit when used as immunizing agents in chronic furunculosis. It is probable that no single protective antitoxic antigen exists, and that the answer lies in polyvalent somatic antigens such as those used by Greenberg and Cooper (1960) in animal experiments or in the teichoic acids, a new class of bacterial antigens discussed by Sanderson *et al.* (1961).

Summary

Alpha-haemolysin and coagulase toxoids and a placebo were given to three groups of patients suffering from chronic furunculosis and their subsequent clinical course was followed for one year.

Statistical analysis showed that neither form of treatment was better than the placebo. A high spontaneous-cure rate was observed.

The significance of alpha-haemolysin and coagulase antibodies before and after immunization and their relation to the clinical state are discussed. It is concluded that immunity to staphylococcal skin infection is independent of these factors, and that the antigenicity of coagulase is almost strain-specific.

It is suggested that antitoxic immunity is of no prophylactic value and that somatic antibacterial immunization may be more effective.

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POST-OPERATIVE HYPOTENSION DUE TO PAROXYSMAL ATRIAL TACHYCARDIA WITH BLOCK

BY

R. M. PRESHAW, M.B., F.R.C.S.Ed.

AND

P. W. C. HARVEY, M.B., M.R.A.C.P.

From the Department of Clinical Surgery, University of Edinburgh ; and the Gastro-Intestinal Unit, Western General Hospital, Edinburgh

In recent years attention has been drawn to paroxysmal atrial tachycardia with block (P.A.T.B.) as a particularly dangerous yet common and often unrecognized complication of digitalis therapy. Despite a description of this arrhythmia by Sir James Mackenzie (1910), it received little attention until the publications of Lown and Levine (1955, 1958).

In this paper we draw attention to possible difficulties in the use of digitalis post-operatively, and describe an example of digitalis-induced P.A.T.B. after gastric surgery in a patient with potassium depletion.

Case Report

The patient, a woman aged 82, had had intermittent dyspeptic symptoms for over 45 years.

Two years ago she was admitted to hospital with acute abdominal pain ; a straight film of her abdomen showed the presence of subdiaphragmatic gas, and she was presumed to have a perforated peptic ulcer. She made an excellent recovery on gastric suction and antibiotics. At that time she complained of minimal exertional dyspnoea but there was no history suggesting coronary insufficiency. She had multiple extrasystoles, her blood-pressure was 190/80, and she had a soft apical ejection murmur. There was no clinical evidence of