

Antibody responses of burned patients immunized with a polyvalent *Pseudomonas* vaccine

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SUMMARY

In two controlled clinical trials of a polyvalent pseudomonas vaccine, vaccinated burned patients showed higher antibody titres to the 16 antigens in the vaccine and higher titres of a passively transferable protective antibody than was found in unvaccinated burned patients.

INTRODUCTION

A new polyvalent pseudomonas vaccine (Miler *et al.* 1977) tested in animals (Jones & Lowbury, 1972) and human volunteers (Jones *et al.* 1976) is undergoing clinical trials on burned patients in Birmingham and New Delhi. The efficacy of the vaccine is being assessed by its capacity to induce useful immune responses and by whether it saves lives (Jones, Roe & Gupta, 1978).

In the trials the immune responses of the burned patients were monitored by a passive haemagglutination test (Jones & Roe, 1975) a passive protection test (Jones, Hall & Ricketts, 1972) and by estimation of IgM and IgG concentrations in serum from burned patients taken at weekly intervals beginning on the day of admission (Jones, Roe & Gupta, 1978).

In a preliminary report on the mortality of burned patients in the vaccine trial in New Delhi (Jones *et al.* 1978) more than half the unvaccinated patients died within 10 days of admission to hospital, while none of the vaccinated patients died, so particular interest was taken in a comparison of immune responses in both vaccinated and unvaccinated patients to see whether humoral antibody played any part in the survival of the vaccinated burned patients.

MATERIALS AND METHODS

Vaccine

Cell wall extracts from a selected strain of *Ps. aeruginosa* from each of the 16 internationally recognized serotypes of *Ps. aeruginosa* (Bergan, 1975) were combined to make a 16-part polyvalent pseudomonas vaccine (Miler *et al.* 1977). The vaccine (Type PEV01) was supplied freeze-dried and suitable for human use by The Wellcome Research Laboratories, it was reconstituted in saline immediately before use.

Burned patients (18–65 years) were injected with 0.5 ml of vaccine subcutaneously on the day of admission, and 7 and 14 days later. Each 0.5 ml of vaccine contained a calculated recommended human dose (Jones *et al.* 1976).

Patients in the trial

In this study patients (18–65 years) with burns of more than 15% whole skin thickness were considered eligible for the trials in Birmingham and New Delhi. Alternate patients were allocated to the vaccinated and control unvaccinated groups.

Treatment of patients

In Birmingham patients were treated prophylactically with silver nitrate chlorhexidine cream (Lowbury, 1976). In New Delhi burns were washed with Savlon on admission, then dressed with vaseline gauze, cotton wool pad and bandage daily.

Immunological tests

A 10 ml heparinized blood sample was collected by venepuncture on the day of admission, 4 and 7 days after admission and then weekly until the patient was discharged. For phagocytic tests (Roe & Jones, 1978) 2 ml of blood was used; the remainder was centrifuged and the plasma collected and stored at 4 °C. In the vaccinated burned patients the blood samples were always taken immediately before vaccination.

Passive haemagglutination test

Each of the 16 component antigens in the polyvalent pseudomonas vaccine (PEV01) were supplied individually by Dr Miler (Wellcome Research Laboratories) and were separately coated onto aliquots of fresh defibrinated sheep erythrocytes (Jones & Roe, 1975).

Plasma (2.5 ml) was heated to 56 °C for 30 min to destroy complement and absorbed with 0.5 ml of washed, packed defibrinated sheep erythrocytes for 30 min to remove sheep erythrocyte agglutinins. Doubling dilutions of the plasma ranging from 1/2 to 1/4096 in 25 μ l amounts using phosphate buffer as diluent were made in plastic trays on an automatic diluter (Titertek). Each sample of plasma was diluted 12 times so that 25 μ l of a 0.2% suspension of sheep erythrocytes sensitized with one of the 16 antigens in the polyvalent vaccine could be added to each of the plasma dilutions from each patient. Agglutination was read after 2 h and overnight at room temperature. A smooth centrally placed button of erythrocytes was taken as being negative; a diffuse spreading of clumps of erythrocytes of irregular sizes was read as positive.

A duplicate set of plasma dilutions was also made of the patient's plasma using plasma that had been pre-treated with 2-mercaptoethanol (Jones *et al.* 1976) to remove IgM. The passive haemagglutination test was performed as stated on these plasma dilutions.

Passive protection tests

Plasma dilutions (1/10, 1/100, 1/500, and 1/1000) in saline of each of the serial samples of plasma from the burned patients were injected in 0.25 ml volumes intraperitoneally into groups of three albino mice (25 g male, BKW₂). Two hours later the mice were challenged intraperitoneally with a saline suspension of *Ps. aeruginosa* P14 (serotype 6) at 1LD100. Control unimmunized mice were also given the same challenge dose of pseudomonas. Deaths were recorded 24 h after challenge.

Immunoglobulin concentrations

Plasma samples from burned patients were examined for IgM and IgG by radial immunodiffusion techniques following the makers (Hoechst, Behring Institute) instructions. The amount of immunoprecipitation was read after 72 h at room temperature.

Bacteriology

Swabs were taken from each burn site on admission and at each change of dressing (usually daily) whenever possible. Swabs were cultured on 4% blood agar and 0.5% cetrinide agar and growth identified according to the methods of Davis, Lilly & Lowbury (1968).

Serotyping of Ps. aeruginosa

All strains of *Ps. aeruginosa* isolated from the burned patients were serotyped according to the methods of Wahba (1965) using rabbit antisera supplied by T. L. Pitt, Cross Infection Laboratories, Colindale, London.

RESULTS

The vaccinated and unvaccinated patients in the Birmingham and in the New Delhi pseudomonas vaccine trials were of similar ages and weights (Table 1) and in both trials the larger burns were found in the groups of vaccinated patients. In Table 1 the main difference between the patients in the two trials was in their weights; the patients in Birmingham averaged 73 kg, while the patients in New Delhi averaged 50 kg.

In the Birmingham trials the predominant serotypes of *Ps. aeruginosa* found on the burns was serotype 11 (Table 2). Slightly more of the unvaccinated patients (50%) were infected with *Ps. aeruginosa* than vaccinated patients (30%). In New Delhi all the patients were infected with *Ps. aeruginosa* at some time during their stay in hospital, the predominating serotypes being 11 and 7. The specific antibodies against the 16 component vaccines in the polyvalent pseudomonas vaccine in patients in Birmingham and New Delhi are shown in Figs. 1, 2, 3 and 4. The antibodies were measured in whole serum and in the IgG fraction of the serum alone.

In whole serum the increase in specific antibody titres in the vaccinated patients

Table 1. *The ages, weights and percentages of body surfaces burned of patients in the pseudomonas vaccine trials in Birmingham and New Delhi*

Treatment group (no. of patients)	Trial	Vaccine dose (RHD)	Age (yr)		EAB (%)		Weight (kg)	
			Range	Mean	Range	Mean	Range	Mean
Vaccinated (9)	Birmingham	1.0	17-56	33	1-60	32	50-95	72.8
Vaccinated (9)	New Delhi	1.0	19-45	30	15-50	29	40-65	50
Unvaccinated (10)	Birmingham	—	19-64	36	15-56	26	50-95	73
Unvaccinated (11)	New Delhi	—	17-46	30	10-40	22	40-65	50

EAB, Estimated percentage of body burned. RHD, Recommended human dose.

Table 2. *Serotypes of Pseudomonas aeruginosa infecting patients with burns in Birmingham and New Delhi during the trial of the pseudomonas vaccine*

Trial	Treatment group	Serotype	Patients with serotypes (%)	Patients colonized with <i>Ps. aeruginosa</i> (%)
Birmingham	Vaccinated	11	100	
		7	10	30
		Ps11	0	
	Unvaccinated	11	100	
		7	0	50
		Ps11	10	
New Delhi	Vaccinated	11	50	
		7	40	100
		022	10	
	Unvaccinated	11	40	
		7	40	100
		022	20	

(continuous line) was greater in the patients in New Delhi than in the Birmingham patients, but in both trials most patients showed rises in antibody against all of the component vaccines. The antibody titres in the unvaccinated patients (broken line) were generally lower than those of the vaccinated patients. On admission most patients had titres of 1/16-1/32 against the majority of the 16 vaccine components and 4 to 5 weeks later titres of 1/2048-1/4096 were recorded against antigens 7 and 8 (Fig. 2), but on average the titres of antibody after 4 to 5 weeks ranged from 1/64-1/256.

The specific antibodies in the IgG fraction of serum (Figs. 3 and 4) in the vaccinated patients showed smaller increases in the patients in the Birmingham trial than in the patients in the New Delhi trial. Against vaccines 9, 10, and 12 in the Birmingham patients there was no change in antibody titre during the 5 weeks shown in Fig. 3; in the control unvaccinated patients rises in IgG occurred against only 5 (vaccines 1, 3, 5, 6, and 11) of the component vaccines. In contrast, the patients in New Delhi showed steadily increasing antibody levels against all 16 antigens in their IgG fractions.

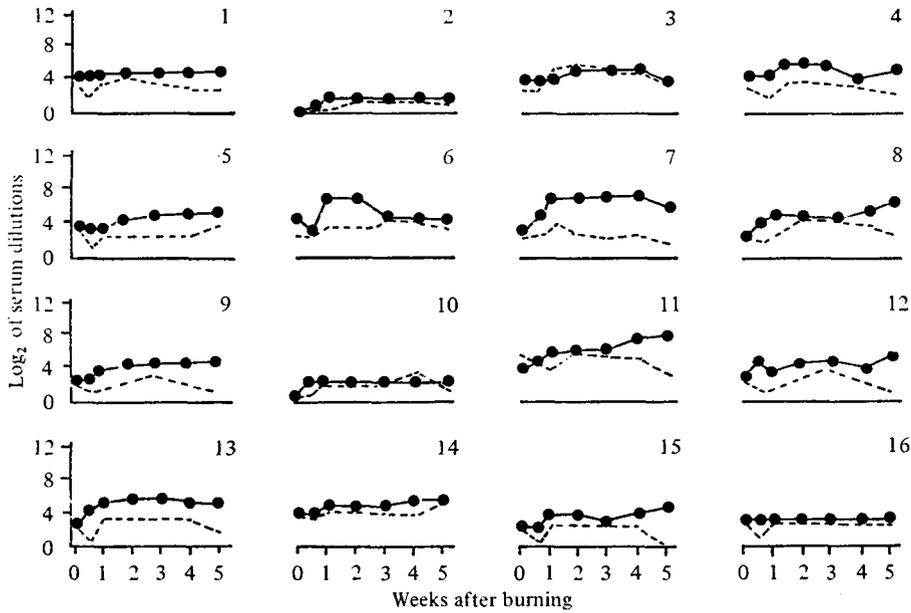


Fig. 1

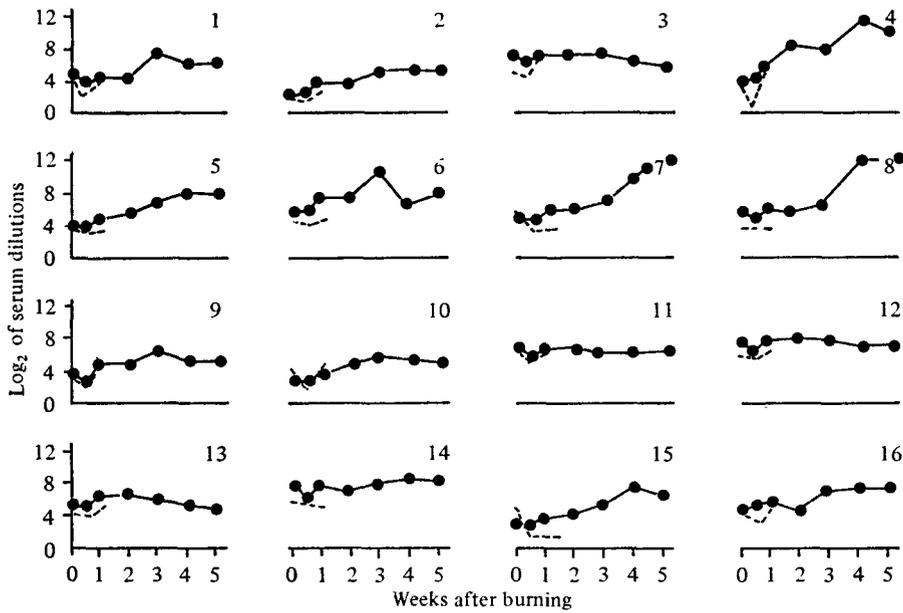


Fig. 2

Figs. 1-4. Specific antibodies against the 16 component vaccines in the polyvalent *Pseudomonas* vaccine in vaccinated (continuous line) and unvaccinated (broken line) burned patients. The antibodies were measured by a passive haemagglutination test in which each component vaccine was coated onto a separate aliquot of sheep erythrocytes. In Figs. 1 and 2, the specific antibodies were measured in serial samples of plasma from burned patients in Birmingham and New Delhi. In Figs. 3 and 4 the specific antibodies were measured in serial samples of plasma containing the IgG fraction of the plasma from burned patients in Birmingham and New Delhi.

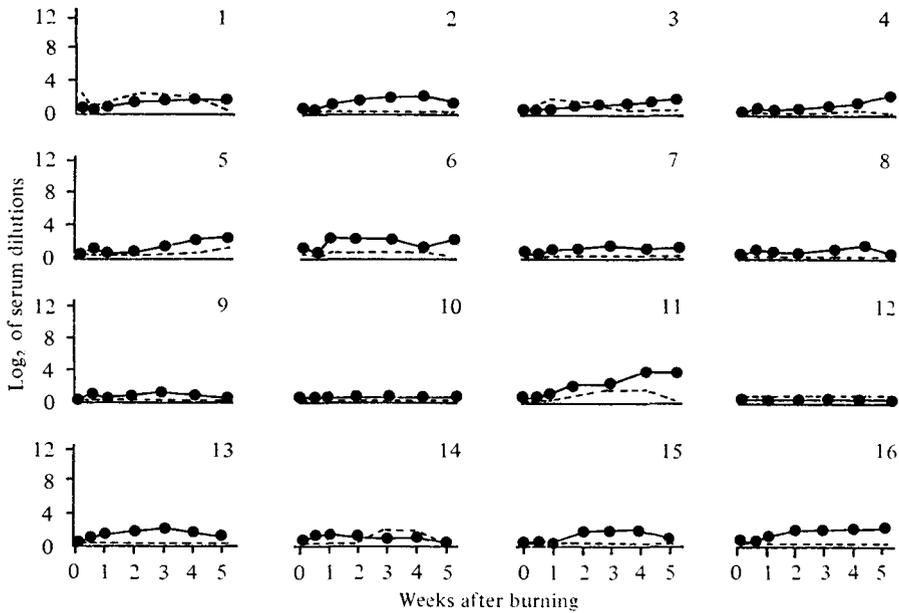


Fig. 3

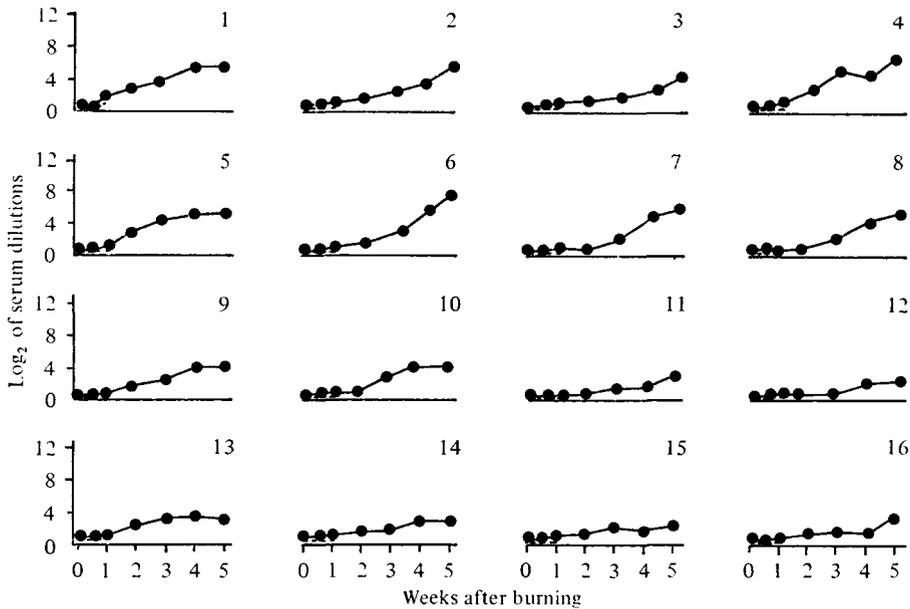


Fig. 4

Passive protective antibodies

In both trials the vaccinated patients showed higher titres of protective antibody in their plasma than the unvaccinated patients (Fig. 5). The peak of protective antibody of the vaccinated patients in Birmingham was higher than in the patients in New Delhi and lasted for a week longer. The titres of protective antibody

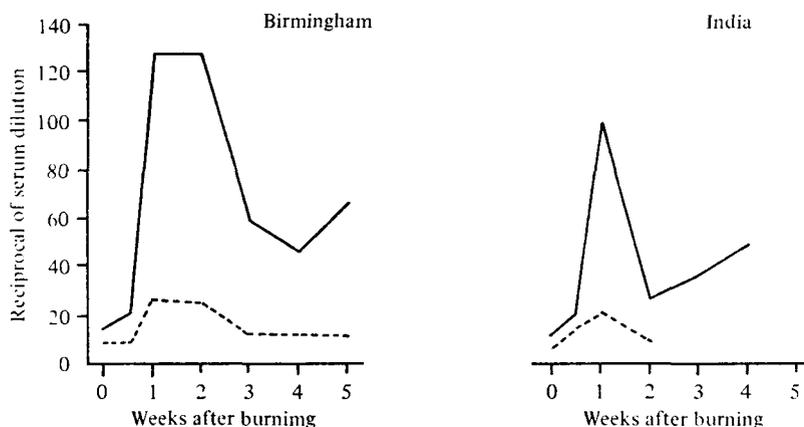


Fig. 5. Comparisons of the passive protective antibody in burned patients in Birmingham and New Delhi. Vaccinated burned patients (continuous line) reached a peak level of protective antibody one week after vaccination; unvaccinated patients (broken line) showed a much smaller rise in protective antibody at this time.

in the vaccinated Birmingham patients fell in the week after the last injection of vaccine (week 2). In the patients in New Delhi the protective antibody titre fell a week before the last injection of vaccine. During the time of the high protective antibody titres in the vaccinated patients there was also a slight increase in those of the unvaccinated patients.

Immunoglobulins M and G

The total immunoglobulin concentrations of IgM and IgG of patients in the Birmingham and New Delhi trials are shown in Figs. 6 and 7. After an initial fall in the IgM during the first week after burning the vaccinated and unvaccinated patients in Birmingham showed a rise in IgM to above the upper range of normal (horizontal broken line) followed by a fall to around mean values of IgM for healthy people. In New Delhi the IgM concentrations of the patients followed the initial pattern set by the Birmingham patients but remained high during the patients' stay in hospital.

The IgG concentrations in vaccinated and unvaccinated patients in Birmingham followed a similar pattern (Fig. 7). After a fall in IgG during the first week after burning, the concentrations rose steadily and remained above the upper limit of the normal value for IgG in healthy people. In contrast, the IgG concentration in the vaccinated patients in New Delhi rose steadily after burning, exhibiting no initial drop after burning. The unvaccinated controls in New Delhi showed a steady fall in IgG until they either died or were discharged.

DISCUSSION

The antibody responses of burned patients in the pseudomonas vaccine trials in Birmingham and New Delhi must be considered against the differences in local conditions prevailing at that time. Both groups of vaccinated patients received

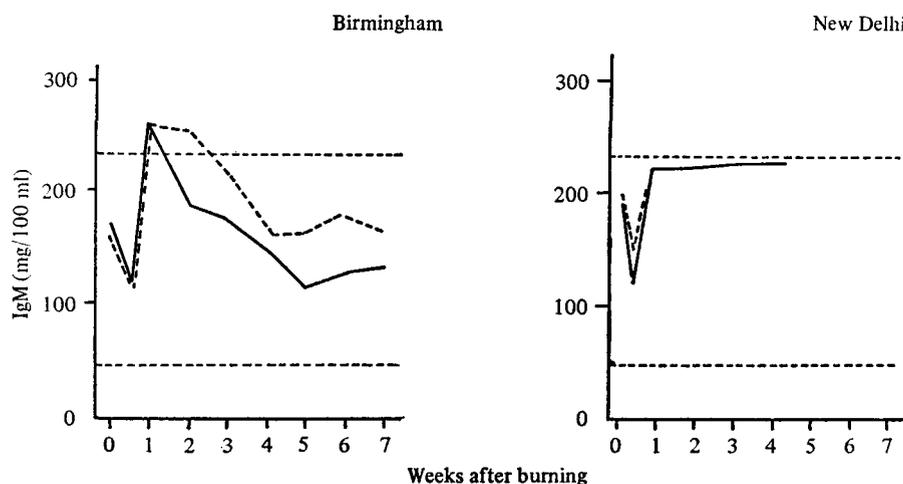


Fig. 6

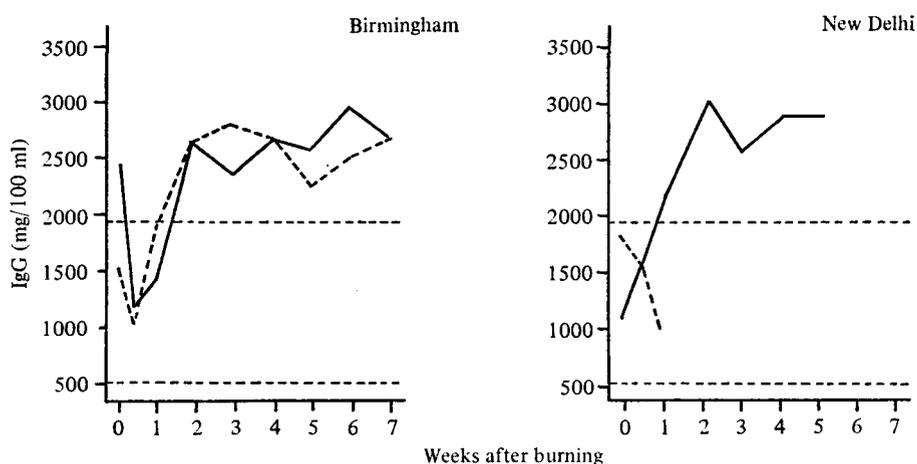


Fig. 7

Figs. 6 and 7. IgM (Fig. 6) and IgG (Fig. 7) concentrations in burned vaccinated (continuous line) and unvaccinated (broken line) patients in Birmingham and New Delhi.

similar doses of vaccine yet the average weights of the patients were vastly different, 50 kg in New Delhi and approximately 73 kg in Birmingham. Thus on a weight basis the Indian patients received a 50% greater dose of vaccine than the patients in U.K. In Birmingham a regime of strict antibacterial prophylaxis exists (Lowbury, 1976) which minimizes and delays the onset of *Pseudomonas* infection: in New Delhi for social-economic reasons the strict antibacterial prophylactic measures are not followed and treatment takes the form of therapy which is ineffective against *Ps. aeruginosa* (Jones, 1974). Patients in New Delhi acquire heavy infections with *Ps. aeruginosa* soon after burning, consequently a greater demand is placed on their immune responses at a time when they are trying to

overcome the metabolic effects of burning. In spite of these disadvantages the patients in New Delhi responded better to the vaccine than the patients in Birmingham. Whether this was due to the extra immunizing power of self infection or to the higher dose of vaccine in proportion to body weight which they received and which has been shown to affect the immunizing potential of the vaccine (Jones *et al.* 1976) has yet to be decided. Self infection as a form of immunization cannot play an important role since the antibody titres in the unvaccinated controls exposed to the same infecting strains of *Ps. aeruginosa* did not rise comparably, if self-infection has any effect on antibody titres it probably takes the form of a booster effect in the patients already primed with at least one injection of vaccine.

The titre of protective antibody in patients also seemed to be influenced by external factors. The low titre of protective antibody found in patients in New Delhi just after the first week of burning occurred at the time when the patients were most heavily infected with *Ps. aeruginosa*. Patients in Birmingham had a lower incidence of infection with *Ps. aeruginosa* during this time and the titre of protective antibody remained high. The utilization of protective antibody for opsonization was shown to be the cause of sharp fluctuations in circulating antibody by Jones & Lowbury (1963).

The passive haemagglutination tests which measure the response of the patients to the 16 component vaccines in the polyvalent vaccine as opposed to measuring the protective function of the patients' antibody, showed that responses to all 16 vaccine components were achieved especially in the IgG fraction of the serum. All patients had specific antibodies in their IgM against all 16 components and these were near to their maximum as further vaccination failed to increase the titres of this antibody. As patients already had antibody to the vaccine a secondary response in the form of a sharp rise in antibody titre might have been expected after vaccination but there was little evidence of this. On the contrary the steady rise in titre in the IgG fraction of the serum indicates a primary response to the vaccine. Possibly if the immunoglobulin concentrations had not fallen during the first week after burning and vaccination then a sharper rise in antibody might have been recorded and would indicate that the antibody response to vaccination could be classed as a secondary response. The results of antibody measurements by different tests show that the passive haemagglutination test measures one type of antibody, presumably responses to the polyvalent vaccine since the individual vaccines are coated onto the erythrocytes, while the passive protection test measures protective antibodies which can be passively transferred. The fall in protective antibody 1–2 weeks after the start of vaccination was not detected by the passive haemagglutination test or by the measurement of total IgM or IgG in the sera. We must therefore conclude that antibodies measured in the passive haemagglutination test are not associated with the passive protective antibodies in burned patients. The passive haemagglutination test therefore only measures responses to the vaccine, which are not associated with protection and results from this test in future must be interpreted with this in mind.

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