PROTECTION AGAINST *PSEUDOMONAS AERUGINOSA* INFECTION BY IMMUNISATION WITH FRACTIONS OF CULTURE FILTRATES OF *PS. AERUGINOSA*

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Received for publication March 6, 1968

THE importance of *Pseudomonas aeruginosa* as a pathogen of extensively burned patients has been shown in many reports (Jackson, Lowbury and Topley, 1951; Markley, Gurmendi, Chavez and Bazan, 1957; Tumbusch, Vogel, Butkiewicz, Graber, Larson and Mitchell, 1961; Rabin, Graber, Vogel, Finkelstein and Tumbusch, 1961; Kefalides, Arana, Bazan, Verlande and Rosenthal, 1964; Jones, Jackson and Lowbury, 1966; Jones and Lowbury, 1967). Even with the methods now available for combating infections by this organism (Moyer, Brentano, Gravens, Margraf and Monafo, 1965; Cason, Jackson, Lowbury and Ricketts, 1966; Jones and Lowbury, 1966, 1967; Lowbury, 1967), it still is reported to cause severe illness and sometimes death from invasive septicaemia (Macmillan, Hill and Altemier, 1967). Of the methods of prophylaxis outlined by Lowbury (1967), the serological method has received very little clinical attention, but recently studies with burned animals (Jones *et al.*, 1966; Jones and Lowbury, 1966) have illustrated the potential usefulness of this method. Other experiments have suggested that patients with low agglutinin levels, or with no detectable agglutinin to *Ps. aeruginosa* in their serum, are more susceptible to pseudomonas invasion than those with higher agglutinin levels (Jones and Lowbury, 1965). The administration of human serum containing raised titres of antibodies to *Ps. aeruginosa*, to patients whose burns were infected with *Ps. aeruginosa*, has been claimed to cause a reduction in mortality from *Ps. aeruginosa* septicaemia (Feller, 1966).

In the studies cited above the antiserum used for passive protection was prepared by inoculation of whole bacterial cells. In 1961, Liu showed that an antiserum prepared against the slime-layer of *Ps. aeruginosa* induced protection against the homologous strain, and more recently Alms and Bass (1966) and Alexander, Brown, Walker, Mason and Moncrief (1966) reported that an alcohol precipitated fraction from a strain of *Ps. aeruginosa* induced protection in mice against challenge by the same organism.

Carney and Jones (1968) have described toxic fractions from virulent and avirulent strains of *Ps. aeruginosa* one of which was of a similar molecular size and type to the immunising fraction described by Alms and Bass (1966).

In the studies reported here extracellular fractions of large molecular size from different strains of *Ps. aeruginosa* were compared for protective activity in burned mice infected with virulent strains of the same and different serotype; extracellular fractions from both virulent and avirulent strains of *Ps. aeruginosa* were studied.

MATERIALS AND METHODS

Strains of Ps. aeruginosa

Two strains of *Ps. aeruginosa* were obtained from the National Collection of Type Cultures: (1) Wahba's Type 14, a sub-type (6C) of serotype 6 referred to as P14; and (2) Veron's Type 2AB (serotype 5C) referred to as P2AB. A third strain (3), B4, was isolated from a patient with burns and was agglutinated by pooled serum from serotype 2 and 5.

Strain P14 and B4 were highly virulent for burned mice; 70 per cent of burned mice infected with a standard inoculum died with *Ps. aeruginosa* septicaemia (Jones and Lowbury, 1967); the mortalities of burned mice infected with P2AB was no higher than that of the burned uninfected mice (about 5 per cent) and none died with septicaemia (Jones and Lowbury, 1966; Jones *et al.*, 1966).

Preparation of immunising fractions

Details of culture, extraction and fractionation have been fully described by Carney and Jones (1968). *Ps. aeruginosa* was grown in a medium in which all constituents were dialysable. The strains were grown for 5 days at 37° in 20 l. volumes in a stainless steel vessel which was aerated by bubbling filtered air through a GS Millipore membrane. After growth, bacteria were removed by centrifugation and positive pressure filtration and the volumes reduced to 250 ml. in a circulatory cyclone evaporator at 25°. The unused constituents of the medium were removed by dialysis. The remaining bacterial products were ultra-filtered to a final volume of 50 ml., centrifuged and lyophilised. The lyophilised material was divided into 4 fractions (F1–F4) on G-200 Sephadex; these fractions were used in the first series of experiments. In later experiments the first fraction from the G-200 Sephadex was further separated on Sepharose-4B into 3 fractions, each of which was assessed for its immunising potential.

Active immunisation of mice with extracellular fractions of Ps. aeruginosa

Protection against homologous strain with fractions from G-200 Sephadex.—Four different dosage levels (10,1, 0·1 and 0·01 mg./kg.) of 3 different extracts of serotype P14 were sterilised by filtration through a syringe fitted Swinny adapter containing a GS Millipore membrane, and inoculated i.p. in 1 ml. saline into groups of 6 male Schofield mice, weighing 10 g. at the beginning of the experiment. The 3 extracts were (1) unfractionated lyophilised material; (2) fraction obtained immediately after the void volume from G-200 Sephadex designated F1 (Carney and Jones, 1968); and (3) fraction F3 containing enzymes (Carney and Jones, 1968). These dosages were administered weekly on 3 occasions. Ten days after the third immunising dose the mice, now weighing nearly 25 g., were depilated by plucking and 4 days later given burns of about 5 per cent of their surface area (Jones and Lawrence, 1964). The surface of the burn was inoculated with a saline suspension containing 7×10^8 organisms of the strain of *Ps. aeruginosa* from which the immunising fractions had been prepared. Mice were maintained as described by Jones and Lowbury (1966).

Protection against homologous and heterologous strains with F1 fractions from G-200 Sephadex.—Thirty mice were inoculated i.p. with 0·1 mg./kg. of *Ps. aeruginosa* strain P14, F1 fraction, and another 30 mice with 0·1 mg./kg. of *Ps. aeurginosa* strain P2AB F1 fraction. The type of mice and immunisation procedure were the same as those described in the experiment above. Ten days after the third immunising dose, 10 mice from each group, after depilation, and burning as before, had the surfaces of their burns inoculated with a saline suspension of *Ps. aeruginosa* strain P14 containing 7×10^8 organisms, another 10 from each group with a similar number of organisms of *Ps. aeruginosa* strain P2AB, and the remaining 10 from each group with *Ps. aeruginosa* strain B4. Unimmunised burned mice, infected with a similar inoculum of the 3 strains of *Ps. aeruginosa* (P14, B4 and P2AB), were included as infection controls. Mice were maintained as previously described.

Protection against homologous and heterologous strains with fractions from Sepharose 4B.—In this experiment the immunising fractions X, Y and Z were obtained by separating (F1) from G-200 Sephadex from *Ps. aeruginosa* strain P14, on a column of Sepharose-4B (Carney and Jones, 1968).

Groups of 20 mice were inoculated with fractions X, Y and Z using similar experimental methods as described for protection experiments with G-200 Sephadex fractions. After the 3 week immunisation course, the mice were depilated and burned as before. The burns of

10 mice from each group were inoculated with 7×10^8 of *Ps. aeruginosa* (P14), the other 10 mice from each group were inoculated with a similar number of organisms of *Ps. aeruginosa* (B4). Ten unimmunised burned mice were infected with strain B4, and a further 10 were infected with strain P14. These two groups of mice served as infection controls.

Passive immunisation of burned mice with antisera to extracellular fractions of Ps. aeruginosa

Antisera to G-200 Sephadex fractions F1 and F3 and to the unfractionated culture filtrate from *Ps. aeruginosa* P14 were raised by i.v. inoculation of rabbits (Carney and Jones, 1968).

Immunisation with antiserum prepared against F1 fraction of Ps. aeruginosa *strain P14.*— One hundred, male Schofield mice (25 g.) were depilated and burned in a standard way (Jones and Lowbury, 1966). Mice, in groups of 20, were each given a single i.p. injection, at the time of burning of either 0·1, 0·01, 0·001, or 0·0001 ml. of rabbit antiserum to fraction F1 from *Ps. aeruginosa* P14. The antiserum was made up to a volume of 1 ml. with saline before injection. The remaining 20 mice were not given antiserum and served as infection controls. Three hr. later, the surfaces of the burns of 10 mice from each group of 20 were inoculated with a saline suspension of *Ps. aeruginosa* P14 containing 7×10^8 organisms; the other 10 mice from each group were inoculated with a similar number of *Ps. aeruginosa* B4.

Immunisation with antiserum prepared against F3 fraction of Ps. aeruginosa *strain P14.*— Groups of 20 mice were each given a single i.p. inoculation of 0·5 ml., 0·1 ml. and 0·01 ml. of antiserum to F3 from *Ps. aeruginosa* strain P14. These mice were then burned and infected as described in the experiment above.

Immunodiffusion

Immunodiffusion techniques described by Carney and Jones, 1968 were used. Serotypes of *Ps. aeruginosa* P1, 2a, 2b, 3, 4, 5c, 5d, 5cd, 6, 7, 8, 9, 10, 11, 12, 13, 14 and strain B4 were grown for 5 days in 20 ml. of dialysable medium (previously described); the bacteria were removed by filtration through a GS Millipore membrane. The filtrate was dialysed, ultrafiltered and freeze-dried. Each culture filtrate was dissolved in 0·4 ml. veronal buffer, pH 8·6 and added to the peripheral antigen holes. The antiserum that was added to the central hole was the same as that used in the passive immunisation experiments (P14 F1). " Z " fraction from P14 was used at 1 per cent concentration.

Agglutination tests

The titration method described by Jones and Lowbury (1965) was used.

RESULTS

Immunising fractions

Culture filtrates of *Ps. aeruginosa* were divided into 4 groups of molecules, on the basis of molecular size, by fractionation on G-200 Sephadex (Carney and Jones, 1968). The 4 groups of molecules were called F1, F2, F3 and F4. F2 and F4 were not used in this study since they were found to have little toxic or enzymic activity (Carney and Jones, 1968). F1 and F3 were selected for study as both F1 and F3 showed some toxicity for mice and F3 contained at least 4 substances, protease, lecithinase, elastase and haemolysin, that are probably concerned in the invasive processes of virulent strains of *Ps. aeruginosa*. It was hoped that by immunisation with sub-lethal dosages of F1 and F3 the severity of the observed toxic effects of the 2 fractions might be reduced. F1 was separated by Carney and Jones (1968) into 3 further groups of molecules (X, Y and Z) on Sepharose-4B and the immunising potential of X, Y and Z was assessed.

Active immunisation of mice with extracellular fractions of Ps. aeruginosa

In Table I, the protective efficacy of active immunisation with F1, F3 and unfractionated culture filtrate from *Ps. aeruginosa* (P14) is shown in groups

TABLE 1.—*Mortality of Burned Mice Immunised with G-200 Sephadex Fractions Prepared from* Ps. aeruginosa (*P14*) *and Challenged with* Ps. aeruginosa (*P14*)

Immunising fraction	Amount given weekly mg./kg.	Number of mice which		Mean survival time of mice which died (days)	*Ps. aeruginosa* in heart blood at autopsy
		Died	Survived		
Unfractionated (P14)	10	4	2	10	4
	1	2	4	9	2
	0·1	4	2	6·5	4
	0·01	4	2	5·5	4
Fraction 1 F1 (P14)	10	4	2	8·5	4
	1	2	4	6·5	1
	0·1	0	6	—	—
	0·01	2	4	1	1
Fraction 3 F3 (P14)	10	3	3	11·3	3
	1	3	3	6·3	3
	0·1	3	3	14·3	3
	0·01	3	3	11	3
None	—	6	6	3·3	6

of mice that were burned and infected with *Ps. aeruginosa* P14, after immunisation. Compared with the unimmunised controls, neither F3 nor the unfractionated material induced protection against septicaemic invasion by *Ps. aeruginosa* (P14); at 1 dosage (0·1 mg./kg.) immunisation with F1 was found to induce protection against homologous challenge. At immunising dosages of F1 > 0·1 mg./kg. the protection against septicaemic invasion was found to be less effective.

Protection of burned mice against homologous and heterologous challenge

Immunisation with G-200 Sephadex fraction F1.—In this experiment an attempt was made to determine the specificity of the protection induced in mice by G-200 Sephadex F1 fraction by:

1. Challenging groups of burned mice, previously immunised with F1 fractions prepared from a virulent strain of *Ps. aeruginosa* (P14), with another virulent strain (B4) of a different serotype *i.e.*, heterologous challenge.
2. Challenging burned mice, previously immunised with an F1 fraction prepared from a strain of *Ps. aeruginosa* (P2AB) that was not virulent for burned mice, with 2 different strains of *Ps. aeruginosa* (P14 and B4) which were highly virulent for burned mice.

It was found (Table II) that during the period when all unimmunised burned mice infected with virulent strains of *Ps. aeruginosa* died, nearly all the mice which had been immunised previously with F1 (0·1 mg./kg.), from either a virulent (P14) or avirulent (P2AB) strain of *Ps. aeruginosa*, were protected from pseudomonas septicaemia and death, irrespective of whether the immunising fraction had been prepared from the infecting strain or not.

At autopsy on the mice which died neither of the 2 which had been immunised with F1 from strain P14 had *Ps. aeruginosa* in their heart blood and death was probably due to peritonitis resulting from a penetrating lesion caused by the burning technique. One of the 2 mice immunised with F1 from strain P2AB was found to have *Ps. aeruginosa* in the heart blood, and the other probably died from peritonitis due to an extension of the burned area into the viscera.

Immunisation with Sepharose-4B fractions.—The G-200 Sephadex F1 fraction from *Ps. aeruginosa* strain P14 was separated into 3 groups of molecules, designated

TABLE II.—*Cross-protection of Burned Mice Infected with Virulent Strains of* Ps. aeruginosa *by Immunisation with G-200 Sephadex Fractions (F1)*

Immunising fraction	Amount given weekly mg./kg.	Strains of *Ps. aeruginosa* inoculated on burns	Number of mice which		Mean survival time of mice that died (days)	*Ps. aeruginosa* present in heart blood at autopsy
			Died	Survived		
Fraction 1 from *Ps. aeruginosa* strain P14	0·1	P14	0	10	—	—
	0·1	B4	1	9	10	—
	0·1	P2AB	1	9	6	—
Fraction 1 from *Ps. aeruginosa* strain P2AB	0·1	P14	2	8	8	1
	0·1	B4	0	10	—	—
	0·1	P2AB	0	10	—	—
Controls						
None	—	P14	5	0	3·8	5
None	—	B4	5	0	6·5	5
None	—	P2AB	0	5	—	—

X, Y and Z in order of decreasing molecular size, after elution through Sepharose-4B (Carney and Jones, 1968). Mice were immunised with these 3 groups of molecules using a similar immunisation schedule to that used for the F1 fraction. It was found (Table III) that all 3 fractions induced similar protection in burned mice which were challenged with either, the strain (P14) from which the immunising material had been prepared, or from a strain (B4) of different serotype. None of the mice which died had *Ps. aeruginosa* in their heart blood and death in all cases was due to peritonitis.

TABLE III.—*Cross-protection of Burned Mice Infected with Virulent Strains of* Ps. aeruginosa *by Immunisation with Sepharose-4B Fractions from* Ps. aeruginosa *Strain P14*

Sepharose fractions used for immunisation	Amount given weekly mg./kg.	Strains of *Ps. aeruginosa* inoculated on burns	Number of mice which		Mean survival time of mice which died (days)	*Ps. aeruginosa* present in heart blood at autopsy
			died	survived		
X	0·1	P14	1	9	9	—
X	0·1	B4	1	9	9	—
Y	0·1	P14	0	10	—	—
Y	0·1	B4	1	9	7	—
Z	0·1	P14	1	9	7	—
Z	0·1	B4	2	8	10	—
Unprotected controls		P14	8	2	3·5	8
		B4	10	0	5·0	10

Passive immunisation of mice with antiserum prepared against extracellular fractions of Ps. aeruginosa

Antiserum prepared against F1, from Ps. aeruginosa, *strain P14.*—Since active immunisation experiments suggested that the Sephadex G-200 F1 fraction contained a factor that protected mice from pseudomonas septicaemia, an antiserum against this fraction was prepared in rabbits, to see if this protection could be administered passively. A single dose of this antiserum, at varying dilutions, was given to groups of 20 mice at the time of burning; 10 mice protected by

serum at each dilution were infected 3 hr. later with the same strain of *Ps. aeruginosa* from which the antiserum had been prepared; the remaining 10 mice in each group were infected with virulent strain—B4 of a different serotype. It was found (Table IV) that the best protection occurred in the group of mice that received 0·1 ml. of serum and which were challenged by the homologous strain (P14); only slight protection was obtained in a group of mice that received the same dose of serum (0·1 ml.) and were challenged by strain (B4).

TABLE IV.—*Passive Immunisation of Burned Infected Mice with Antiserum Prepared from the F1 Fraction of* Ps. aeruginosa *Strain P14*

Material used for preparing antiserum		Amount of antiserum given (ml.)	Strain of *Ps. aeruginosa* used for challenge	Number of mice which		Mean survival time of mice which died (days)	*Ps. aeruginosa* in heart blood at autopsy
Strain of *Ps. aeruginosa*	Fraction			Died	Survived		
P14	F1	0·1	P14	1	9	14	1
		0·01	P14	3	7	9·8	1
		0·001	P14	9	1	4·6	9
		0·0001	P14	8	2	3·2	8
P14	F1	0·1	B4	6	4	7·5	6
		0·01	B4	9	1	6·3	9
		0·001	B4	9	1	3·3	9
		0·0001	B4	10	—	4·0	10
Unprotected controls			P14	10	—	3·5	10
			B4	10	—	4·2	10

Antiserum prepared against the F3 fraction of Ps. aeruginosa, *strain P14.*—In this experiment groups of burned mice were infected with different virulent strains of *Ps. aeruginosa* after being given a single inoculation, at the time of burning, of an antiserum prepared by immunising rabbits with the F3 fraction from *Ps. aeruginosa* strain P14. The effect that varying dilutions of this serum had in reducing mortality from invasive septicaemia, in groups of mice which had received this serum and which were challenged with the homologous or heterologous virulent strains of *Ps. aeruginosa*, is shown in Table V. It was found that the antiserum prepared against the F3 fraction induced a slightly greater protection in the group of mice challenged with strain P14 (homologous strain), than in the group of mice challenged with strain B4 (heterologous strain).

TABLE V.—*Passive Immunisation of Burned Infected Mice with Antiserum Prepared from the F3 Fraction of* Ps. aeruginosa *Strain P14*

Material used for preparing antiserum		Amount of antiserum given (ml.)	Strain of *Ps. aeruginosa* used for challenge	Number of mice which		Mean survival time of mice which died (days)	*Ps. aeruginosa* in heart blood at autopsy
Strain of *Ps. aeruginosa*	Fraction			Died	Survived		
P14	F3	0·5	P14	2	8	8	2
		0·1	P14	3	7	8	3
		0·01	P14	5	5	5·2	5
P14	F3	0·5	B4	4	6	11	4
		0·1	B4	4	6	5·7	4
		0·01	B4	7	3	7·5	7
Unprotected controls			P14	8	2	4·5	8
			B4	5	5	5·0	5

Immunodiffusion

Freeze-dried culture filtrates of 18 different serotypes and subtypes of *Ps. aeruginosa* and a culture filtrate from a strain isolated from a burned patient (B4) were diffused against an antiserum prepared in rabbits against the F1 fraction of *Ps. aeruginosa* P14, (Figs. 1, 2 and 3). The precipitation patterns of the culture filtrates were compared with precipitation pattern of the protective fraction " Z ", from strain P14, which Carney and Jones, 1968 showed to be a relatively non-toxic fraction. All culture filtrates, except P4, P7 and P5CD, were found to have antigenic factors in common with P14, " Z " fraction.

Agglutination of serotypes of Ps. aeruginosa *by antiserum to* Ps. aeruginosa *P14, F1 fraction*

Table VI shows the titres of agglutinin to 19 different strains of *Ps. aeruginosa* in serum prepared in rabbits to F1 from strain P14. Only 4 (1, 8, 11 and 13) of the 19 strains were found to have low titres of agglutinins in this serum; the majority of the strains showed titres of 3200 and above.

TABLE VI.—*Titre of Agglutinins (to Formolised Suspensions of Different Serotypes of* Ps. aeruginosa) *in Antiserum Prepared Against F1 from* Ps. aeruginosa *(P14)*

Serotype of *Ps. aeruginosa*	Reciprocal of titre of agglutinins to *Ps. aeruginosa* in P14, F1 A/S
1	1
2A	3200
2B	3200
2AB	6400
3	6400
4	4800
5C	6400
5D	12,800
5CD	1600
6	3200
7	3200
8	1
9	6400
10	3200
11	1
12	3200
13	4
14	12,800
Strain B4	9600

The titres of agglutinin to strains B4 and P14 used for challenge in the passive immunisation experiments with this serum (Table IV) were 9600 and 12,800 respectively.

DISCUSSION

Burned mice infected with virulent strains of *Ps. aeruginosa* were protected from septicaemia and death by active immunisation with culture filtrates of *Ps. aeruginosa* during a 3 week period before burning and infection.

The immunising fractions that induced protection in mice were prepared by gel filtration of non-dialysable culture filtrates of *Ps. aeruginosa* on G-200 Sephadex

and Sepharose-4B using fractionation methods described by Carney and Jones (1968). Only 1 (F1) of the 4 fractions separated from culture filtrates by G-200 Sephadex, induced active protection in mice. The protective immunising dosage of F1 was 0·1 mg./kg. Protection was not as good in groups of mice immunised with dosages of F1 that were greater than 0·1 mg./kg., probably because the higher immunising dosages (1 and 10 mg./kg.) were approaching the LD 50 for P14 F1, which is in the region of 17·5 mg./kg. (Carney and Jones, 1968.) When F1 was divided into 3 further fractions (X, Y and Z) by Sepharose-4B; all 3 fractions were found to induce protection. This last observation is of some importance if these fractions are to be considered for use in active immunisation against *Ps. aeruginosa* infection, since the Z fraction was shown by Carney and Jones (1968) to be non-lethal for mice at 40 mg./kg. (*i.e.* a dosage 400 times that of the immunising dosage (0·1 mg./kg.) did not kill mice).

Most of the infected actively immunised mice that died, did so as a result of an extension of the burned area to the organs in the peritoneal cavity and not from invasive septicaemia. Variation in the depth of dorsal burns in rats was found by Zawacki and Jones (1967), who used the same burning apparatus as used in these experiments, to be associated with regional variation in skin thickness due to the hair growth cycle. A similar hair growth cycle also occurs in mice (Montagna, 1962) and burning on thin areas of skin could account for the penetrating effects produced by some of the burns.

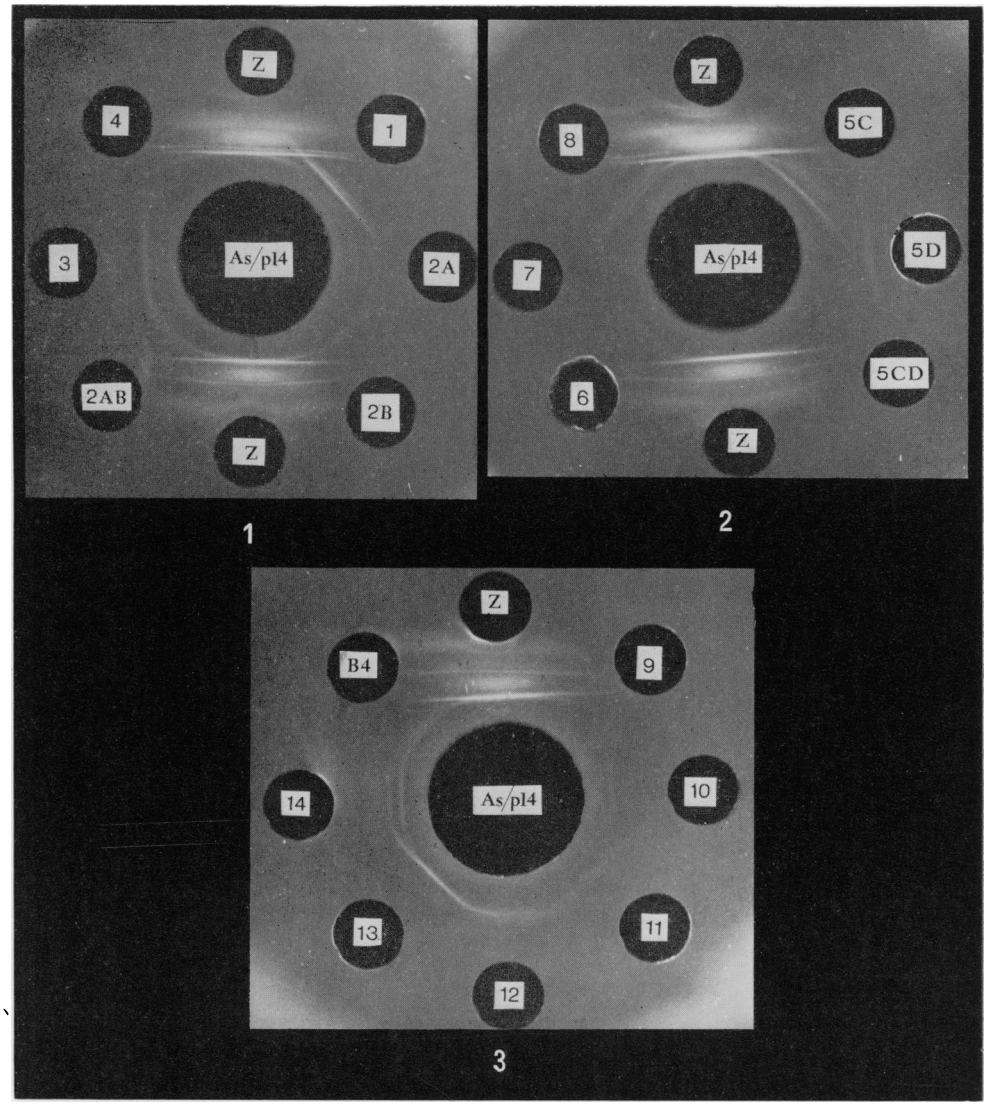
The protection induced by immunisation of mice with F1 fractions was found to be as effective when the immunising fractions were derived from a serotype other than that of the infecting strain as when the immunising fractions were derived from the infecting strain of *Ps. aeruginosa*. In one experiment protection was obtained against challenge from 2 highly virulent strains by immunising mice, before burning and infection, with a fraction from an avirulent strain that was of a different serotype from that of the 2 infecting strains.

It is not known against how many serotypes of *Ps. aeruginosa* each F1 fraction will protect. Jones and Lowbury (1965) found that the presence of pseudomonas agglutinins in serum was an index of the protective capacity of the serum. If the agglutination titres of 19 different strains of *Ps. aeruginosa* in antiserum to P14 F1 can be regarded as an indication of the range of serotypes against which each F1 fraction would protect, then F1 fractions from very few serotypes would be needed to induce protection against all strains of *Ps. aeruginosa*, provided that all strains of *Ps. aeruginosa* produce protective factors in their F1 fractions.

In pilot studies using immunodiffusion techniques, antigenic factors common to fraction Z—a non-toxic protective fraction—and to most of the culture filtrates of 19 different strains of *Ps. aeruginosa* were found, and provide further evidence of a serological relationship between protective fractions and different serotypes of *Ps. aeruginosa*.

EXPLANATION OF PLATE

FIGS. 1, 2, and 3.—Comparison of precipitation patterns of extracellular fractions from, 18 different serotypes and subtypes of *Ps. aeruginosa* and a strain of *Ps. aeruginosa* (B4) isolated from a patient with burns, with a protective fraction " Z " from a virulent strain of *Ps. aeruginosa* (P14).
The antiserum was prepared against a G-200 Sephadex fraction (F1) that contains protective factors.


Z
4
1
As/pl4
3
2A
2AB
2B
Z
1
Z
8
5C
As/pl4
5D
7
6
5CD
Z
2
Z
B4
9
As/pl4
14
10
13
11
12
3

Jones.

Passive immunisation of mice at the time of burning with an antiserum prepared in rabbits against the (F1) fraction suggested that the protection passively induced in the mice might be specific only for the strain against which the protective antiserum had been prepared, since better protection was obtained against challenge by the homologous infecting strain (the strain from which the immunising fractions had been prepared) than against challenge by a heterologous virulent strain. However, the apparent lack of protection of burned mice by passively administered serum against challenge from heterologous strains of *Ps. aeruginosa* can probably partly be explained by deficiencies in the amount or frequency of the serum given to burned mice, as well as by the absence of cross-protecting antibodies in the serum.

The protective fractions described above are chemically more homogenous than bacterial cells and could be used to raise hyperimmune sera in animals or man. Such hyperimmune serum would be useful for passive prophylactic or therapeutic treatment of *Ps. aeruginosa* infection. Alternatively, in appropriate cases, where patients are in hospital prior surgery, they could be actively immunised with non-toxic protective fractions to raise their protective antibody levels before they were at risk to infection with *Ps. aeruginosa*. It would probably be in the field of organ transplantation that active immunisation would prove to be most useful; since it should be possible to induce a high level of protection against *Ps. aeruginosa* in the patient before immunosuppression; some serum could be removed from the patient and kept in store for passive protection at a later date, should the need arise.

Experiments were also made with burned mice passively immunised with an antiserum prepared in rabbits against F3, a G-200 Sephadex fraction containing a wide range of enzymes with potential importance in pathogenicity; these experiments showed that the protection obtained with this serum was less specific than that obtained with antiserum prepared from F1. This non specific protection induced by F3 antiserum suggests that some factors in the F3 fractions of virulent strains were probably of importance in establishing a fatal infection in mice, since it was possible to reduce the number of deaths from pseudomonas septicaemia by antagonising the factors with F3 antiserum. Whether antiserum to P14 F3 reduced the mortality of the infected burned mice by antagonising the enzymes in F3 has still to be proved.

It is hoped to extend this work to include other Gram-negative organisms, and to develop an *in vitro* test for the titration of protective antibodies. Since the extracellular enzymes of *Ps. aeruginosa* appear to play a role in establishing fatal infections, more work on specific antagonists of these enzymes, whether chemical or serological, might help to reduce the severity of local lesions and reduce the likelihood of pseudomonas septicaemia.

SUMMARY

Burned mice challenged with virulent strains of *Ps. aeruginosa* after active immunisation with large molecular fractions (F1) of culture filtrates of *Ps. aeruginosa*. were protected against pseudomonas septicaemia and death. Mice immunised with F1 from one serotype of *Ps. aeruginosa* were protected against infection by 2 other serotypes of *Ps. aeruginosa*. F1 from an avirulent strain induced active immunity.

Passive immunisation of mice, at the time of burning and 3 hr. before infection, with an antiserum prepared in rabbits to F1 from a single strain of *Ps. aeruginosa* (P14) gave better protection against the strain from which the antiserum had been prepared than against challenge from another virulent serotype of *Ps. aeruginosa*. In similar experiments, passive immunisation with antiserum, prepared in rabbits from a fraction (F3) of a culture filtrate of *Ps. aeruginosa* that contains the majority of extracellular enzymes of *Ps. aeruginosa*, gave less specific protection than antiserum to F1 fractions.

The possible role of these fractions in active and passive immunisation of patients at risk with pseudomonas sepsis is discussed.

I wish to thank Dr. B. T. Thom, Central Public Health Laboratories for serotyping the strains of *Ps. aeruginosa* used, Dr. E. J. L. Lowbury for useful discussions and R. E. Dyster and P. Iremonger for technical assistance.

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