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Tests for Penicillin Allergy in Man

II. THE IMMUNOLOGICAL CROSS-REACTION BETWEEN PENICILLINS AND CEPHALOSPORINS

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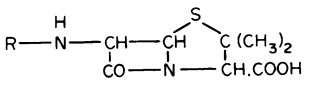
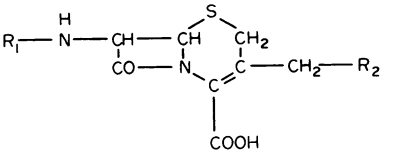
Summary. The immunological cross-reaction between penicillins and a cephalosporin derivative (cephaloridine CEPR) has been investigated. Cross-allergenicity was studied in twenty-four patients with established penicillin allergy using a variety of tests. Skin tests, quantitative leucocyte challenge (estimating histamine release by allergen) and lymphocyte stimulation (transformation) tests were performed, using CEPR, conjugates of CEPR with human serum albumin (HSA) and bovine gamma-globulin (BGG), benzylpenicillin and benzylpenicilloyl (BPO) conjugates with HSA and BGG. A cross-reaction was clearly established in the majority of patients. The highest percentage of positive results (cross-allergenicity) was obtained in the leucocyte challenge test (80 per cent with CEPR.BGG), followed by the lymphocyte stimulation test (50 per cent with CEPR.BGG), and then the skin test (46 per cent with CEPR.HSA). None of ten non-allergic controls gave a positive result in these tests. Three of the penicillin allergic patients had received cephaloridine, and all three developed allergic reactions. Cross-antigenicity has also been shown by haemagglutination and haemagglutination-inhibition tests on serum from larger groups of penicillin-allergic patients (including the previously mentioned twenty-four patients), and of non-allergic controls, who had anti-BPO antibodies.

INTRODUCTION

The group of antibiotics derived from cephalosporin C was introduced several years ago, and it was hoped that it would be sufficiently different from penicillin to avoid any immunological cross-reaction. The cephalosporins differ from the penicillins in that the five-membered thiazolidine ring of penicillin is replaced by a six-membered dihydrothiazine ring.



Penicillins



Cephalosporins

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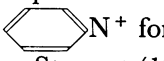
The side chain structures of most of the cephalosporins available now also differ from those of penicillins, e.g. benzylpenicillin has as a side-chain phenylacetic acid, whereas both cephaloridine (CEPR) and cephalothin (CEPT) have R_1 side chain thiophene acetic acid. On the other hand, the R side-chain of ampicillin is similar to the R_1 of cephalexin. The cephalosporins have further potential for variety via the R_2 side-chain;  N^+ for cephaloridine (CEPR) and CH_3COOH for cephalothin (CEPT).

Stewart (1962) reported that cephalosporin C was not cross-allergenic with the penicillins in skin tests in man, and several reports have indicated that cephalosporins can safely be taken by patients sensitive to penicillin (Griffith and Black, 1964; Marks and Garrett, 1970; Stewart, 1967; Weinstein, Kaplan and Chang, 1964). Schneirson, Perlman and Shore (1964) reported that CEPT can stimulate the production of immunological reactions in rabbits and guinea-pigs, but they found no evidence of cross-reaction with penicillin.

In contrast, clinical reactions (mostly severe anaphylactic shock) on first exposure to cephalosporins have been reported in patients sensitive to penicillin (Kabins, Eisenstein and Cohen, 1965; Rothschild and Doty, 1966). In man cross-allergenicity and cross-antigenicity have been demonstrated using skin tests (Grieco, 1967; Girard, 1968), and haemagglutination studies (Molthan, 1968), and in rabbits this has been shown by inhibition of precipitation (Brandriss, Smith and Steinman, 1965), haemagglutination and PCA reactions (Batchelor, Dewdrey, Weston and Wheeler, 1966). The penicilloyl group seems to be largely responsible for the cross-reaction (Ky, Chauvin, Pinon and Halpern, 1970), and it has been suggested that the β-lactam ring of cephalosporins may split, giving a cephalosporoyl group with areas of close structural similarity (Feinberg, 1968). The R_1 side-chain also seems to play an important role in the cross-reaction, e.g. between benzylpenicillin (PenG) and cephalothin, and ampicillin and cephalexin (Shibata, Atsumi, Horiuchi and Mashimo, 1966; Mashimo, 1969).

We have studied the cross-reaction between penicillins and cephaloridine in man, both in allergic patients and in a large group of non-allergic controls, using a variety of techniques.

MATERIALS AND METHODS

Patients

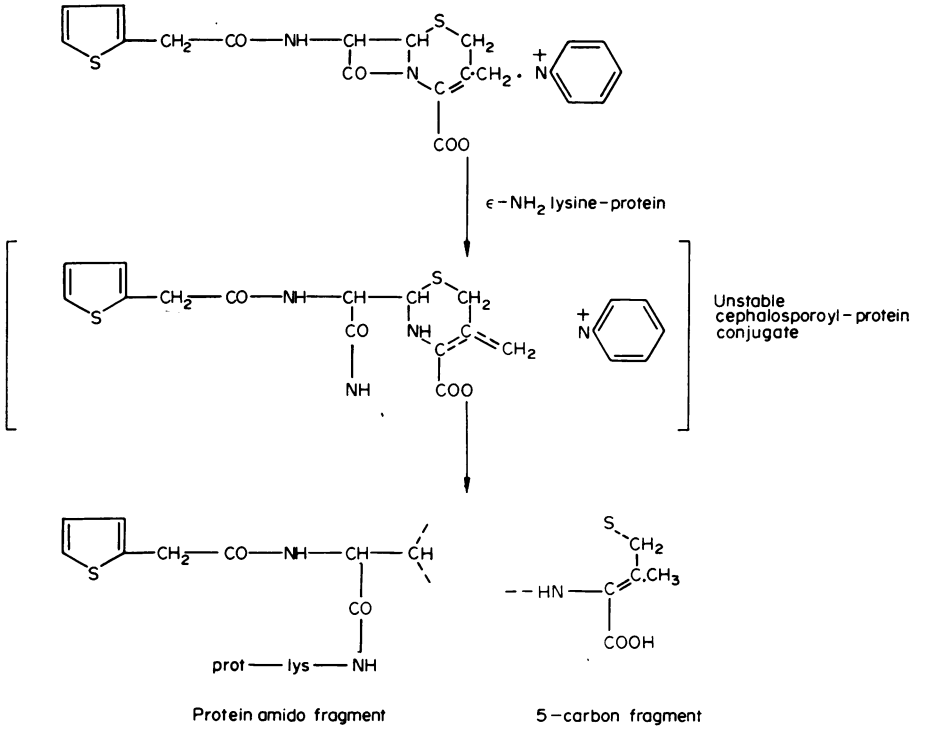
Twenty-four patients whose allergy to penicillin was clinically well documented and proven by a variety of *in vivo* and *in vitro* tests were selected for the study of cross-allergenicity. Three of these patients had received cephaloridine. All three developed allergic reactions to cephaloridine, one (number 15) had an anaphylactic reaction, one (number 2) developed extensive urticaria, and the third (number 13) a serum sickness-like syndrome. Ten control subjects with no clinical evidence of penicillin allergy, and who have been similarly investigated, were also included. Serum from a larger group of penicillin-allergic patients (thirty-nine) and from thirty-five subjects with no personal or family history of penicillin allergy, all of whom had received penicillin but not cephalosporins, were also examined for anti-penicilloyl and anti-cephaloridine antibodies.

Antigens

Benzylpenicillin and cephaloridine ('Ceporin') were obtained from Glaxo Laboratories, Greenford, England. Cephalothin was obtained from Eli Lilly and Company.

Benzylpenicilloyl protein conjugates were prepared by the method described by Parker and Thiel (1963). Benzylpenicillin was coupled directly to the protein by reaction with protein amino groups at pH 11·0. The degree of substitution of the protein by penicilloyl groups was estimated using the penamaldate assay described by Levine (1962). The preparations were: benzylpenicilloyl$_{14}$–HSA and benzylpenicilloyl$_{12}$–BGG.

The cephaloridine–HSA and cephaloridine–BGG conjugates were prepared by reaction of the protein in aqueous solution at high pH. A cephalosporoyl protein conjugate similar to the penicilloyl–protein conjugate would be expected to form by direct aminolysis of the protein (Feinberg, 1968). It has been suggested, however, that the proposed 'cephalosporoyl–protein' conjugate would be unstable and would rapidly fragment (Newton and Hamilton-Miller, 1967), as illustrated in the following diagram.



The absorption maximum of cephaloridine is 260 nm, and the preparations were estimated by comparing their absorption at 260 nm with that of a series of standard preparations. This method of estimation was thought to be the best in view of the uncertainty concerning the chemical nature of the haptenic groups, even though the absorption maximum of the haptenic groups formed must differ from that of cephaloridine itself. The preparations were cephaloridine$_{11}$–HSA and cephaloridine$_{10}$–BGG.

Skin tests

Skin tests were carried out by intradermal injection of 0·02 ml of antigen solution. Benzylpenicillin (B.Pen.) was given in concentrations of 100 and 1000 u/ml; cephaloridine (CEPR) in concentrations of 0·1 and 1·0 mg/ml; benzylpenicilloyl–HSA (BPO.HSA) and cephaloridine–HSA(CEPR.HSA) in concentrations of 1, 5 and 25×10^{-5}M (with

respect to the haptenic group). The diameters of the wheal and flare reactions were measured after 15 minutes, and patients were asked to report any reactions which developed later. The reaction was considered positive when the diameter of the wheal was greater than 5 mm and the diameter of the flare greater than 10 mm.

The release of histamine from sensitized human leucocytes

This test is an *in vitro* correlate of immediate-type allergy. The method used to isolate the leucocytes was that described by Assem and McAllen (1970), which is a modification of the technique of Lichtenstein and Osler (1964). Leucocytes were isolated from heparinized venous blood, and challenged with antigen or with Tyrode solution (as a control). B.Pen. was used in concentrations of 10, 100 and 1000 u/ml; CEPR in concentrations of 0·04, 0·2 and 1·0 mg/ml; CEPR.BGG and BPO.BGG in concentrations of 1, 5 and 25×10^{-5}M (with respect to the haptenic group). The histamine released and the residual cell histamine were measured by bio-assay on guinea-pig ileum (Assem and Schild, 1968) and the released histamine expressed as a percentage of the total histamine (released + residual). A positive result was taken as a percentage histamine release from antigen-challenged leucocytes equal to twice that from Tyrode-challenged leucocytes.

The lymphocyte transformation test (stimulation)

This test gives positive results in cases of immediate and delayed-type allergy. The technique used to separate lymphocytes from peripheral blood was that described by Coulson and Chalmers (1967) and DNA synthesis was measured as described by Chalmers, Cooper, Evans and Topping (1967). Venous blood samples were defibrinated and lymphocytes separated. The lymphocytes were suspended in tissue culture medium 199 (Wellcome Laboratories) containing 10 per cent autologous serum and divided into 2·7-ml aliquots. Antigen or medium (0·3 ml) was added and then the cells incubated for 4 days at 37°C. After the incubation the incorporation of [^{3}H]thymidine over a 1-hour period was measured.

CEPR was used in final concentrations of 0·4, 2·0 and 10·0 μg/ml; B.Pen. in concentrations of 10 and 100 u/ml; CEPR.BGG and BPO.BGG in concentrations of 1, 5 and 25×10^{-6}M.

This test was considered positive when the counts per minute (cpm) of the antigen-challenged samples were significantly higher than the cpm of the control samples. Four samples of each type were prepared, and the significance of the difference between various treatments calculated from Student's *t*-test.

Haemagglutinating antibodies

Preparation of sheep red blood cells coated with antigen. The method used was basically that of Fulthorpe, Roitt, Doniach and Couchman (1961), which involves tanning the cells, coating with antigen and then adding 40% formalin to stabilize and preserve the cell–antigen preparation. The formalinized cells are allowed to stand for a few days and are then washed in borate–succinate buffer, 0·05 M, pH 7·5, and finally made up to a 1 per cent suspension in borate–succinate buffer with 0·2 per cent formalin added as preservative. It was found, however, that considerable haemolysis occurred during the addition of 40 per cent formalin and also during the washing with borate–succinate buffer. To overcome these difficulties the cells were formalinized before tanning or coating with antigen, and thiomersalate was added to the buffer as preservative. With this addition the pH of

the borate–succinate buffer remained stable throughout the course of the experiment, and little haemolysis occurred during the washing stages.

Preparations of sheep red blood cells (SRBC) coated with HSA, penicilloyl–HSA, and cephaloridine–HSA, were made.

Passive haemagglutination procedure. The serum to be tested was diluted one in five with HSA-coated SRBC and left overnight at 4°C. The cells were then spun down and the serum removed. This procedure removes antibodies in the serum which react non-specifically. Haemagglutination tests were carried out using a Takatsy microtitrator,

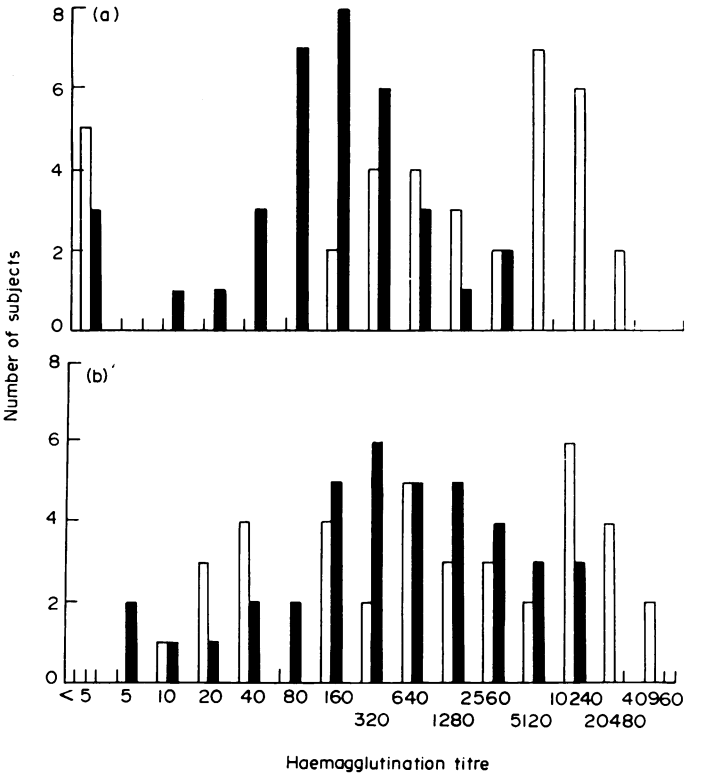


FIG. 1. Haemagglutination of sheep red blood cells (SRBC) coated with penicilloyl–human serum albumin (HSA) conjugate (□), and of SRBC coated with cephaloridine–HSA conjugate (■) by (a) serum from normal subjects, and (b) from penicillin-allergic patients.

dropping pipette and plexiglass block. Two-fold dilutions of the serum were made in 0·6 per cent w/v dextran (M.W. 110,000) in 0·9 per cent saline, and an equal volume of SRBC coated with either BPO.HSA or CEPR.HSA was added to each well. For each serum a control was set up with HSA-coated cells. The contents of the wells were mixed by gentle rotation and left at room temperature for 3 hours for the agglutination pattern to develop. The titre of the serum was taken as the last dilution, which gave a clearly defined agglutination of the cells.

Absorption of serum with BPO.HSA-coated cells. To study the cross-reaction between BPO and CEPR a selection of eighteen serum samples from penicillin allergic patients was

taken and incubated overnight with SRBC coated with BPO.HSA. The cells were spun down and the diluted serum used for for haemagglutinatin.

RESULTS

HAEMAGGLUTINATING ANTIBODIES

Haemagglutinating antibodies specific for penicilloyl and for cephaloridine were found in the majority of penicillin-allergic and control subjects tested. The spread of titres is shown in Fig. 1. The titre for CEPR is generally lower than that for BPO. Taking a titre of

TABLE 1

PENICILLOYL AND CEPHALORIDINE HAEMAGGLUTINATION TITRES IN SOME PENICILLIN-ALLERGIC SUBJECTS

Patient number	Haemagglutination titre		
	BPO	CEPR	CEPR after BPO absorption
1 SA	10241	2560	<5
2 CC†	640	160	<5
3 JC	40960	5120	20
4 RC	2560	640	<5
5 GI	160	10240	320
6 FM	10240	2560	20
7 NS	160	1280	80
8 SS	640	1280	10
9 JS	10240	1280	10
10 DS	40960	10240	<5
11 MR	20480	5120	40
12 ML	30	120	<5
13 EW†	2560	640	<5
14 AW	360	360	10
15 JW†	20480	10240	320
16 CT	20480	5120	<5
17 FH	160	160	20
18 LC	20	20	<5

Note the fall in the cephaloridine-specific haemagglutination titre after the serum has been absorbed with penicilloyl–HSA.

† Had received and reacted to ceporin.

20 as positive, thirty-six out of thirty-nine penicillin allergic patients had antibodies which agglutinate CEPR-coated SRBC compared with thirty-eight out of thirty-nine who had BPO-specific antibodies. In the non-penicillin allergic group thirty-one out of thirty-five had positive CEPR titres compared with thirty out of thirty-five with positive BPO titres.

When BPO-specific antibodies in eighteen sera were absorbed and the serum then tested for agglutination of CEPR-coated cells the CEPR titres were greatly reduced in all cases (Table 1) and in eight cases complete inhibition was seen.

SKIN TESTS

In the penicillin allergic group eleven out of twenty-four (46 per cent) gave positive skin reactions to CEPR.HSA and eleven out of twenty-four (46 per cent) to CEPR (Table 2).

Five patients responded to CEPR and not to CEPR.HSA, and five responded to CEPR. HSA in the absence of a response to CEPR. One of the patients who had received CEPR and developed an anaphylactic reaction failed to give a positive skin reaction

TABLE 2

SKIN TESTS, HISTAMINE RELEASE FROM SENSITIZED LEUCOCYTES AND THE LYMPHOCYTE TRANSFORMATION TEST IN PENICILLIN-ALLERGIC AND IN CONTROL SUBJECTS

Test system	Penicillin-allergic				Not penicillin-allergic			
	CEPR	CEPR HSA/BGG	B.Pen	BPO HSA/BGG	CEPR	CEPR HSA/BGG	B.Pen	BPO HSA/BGG
Intradermal skin tests	11/24 (46%)	11/24 (46%)	7/24 (29%)	11/24 (43%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
Leucocyte challenge tests	7/20 (35%)	16/20 (80%)	6/20 (30%)	15/20 (75%)	N.D.	0/10 (0%)	N.D.	1/10 (10%)
Lymphocyte transformation tests	8/20 (40%)	10/20 (50%)	8/20 (40%)	14/20 (70%)	N.D.	0/10 (0%)	N.D.	1/10 (10%)

N.D. = not determined.

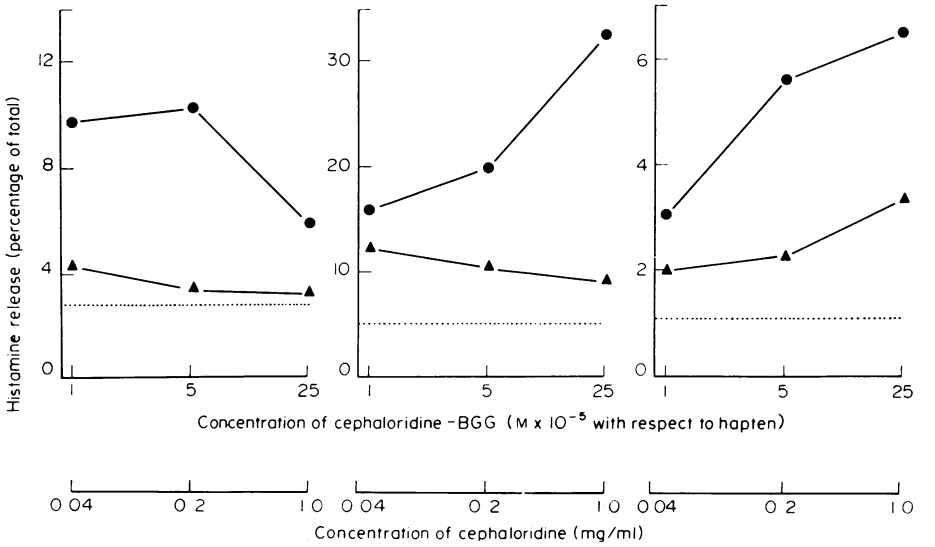


FIG. 2. Leucocyte challenge test (histamine release) in three penicillin-allergic patients, carried out in the presence of cephaloridine (▲), and in the presence of cephaloridine–bovine gamma-globulin conjugate (●). (– – –) Control.

to CEPR or to CEPR.HSA in the following 2 months, but no further follow-up of the skin test has been carried out.

There was no apparent correlation between the skin response to penicillin, and that to cephaloridine. The number of positive responses was equal for BPO.HSA (eleven out of twenty-four), but lower for B.Pen (seven out of twenty-four). No positive skin reactions were obtained in the ten control subjects tested.

Leucocyte challenge test

In the penicillin allergic group the number of patients responding to CEPR itself was low, seven out of twenty (35 per cent) but when the CEPR.BGG conjugate was used positive results were obtained in sixteen out of twenty patients (80 per cent) (Table 2). In comparison six out of twenty responded to B.Pen (30 per cent) and fifteen out of twenty (75 per cent) to BPO.BGG. The highest response for CEPR.BGG was generally obtained at 25×10^{-5}M as compared with 5×10^{-5}M for the BPO.BGG conjugate. Some typical dose response curves are shown in Fig. 2.

Three patients responded to CEPR.BGG in the absence of a response to either BPO. BGG or to B.Pen. Two of these three patients had received and reacted to ceporin. A further five patients gave a greater response to CEPR. BGG than to BPO.BGG although in one of these cases (number 16) the response to B.Pen was greater than that to CEPR. BGG.

In the non-allergic group no positive responses were obtained with CEPR.BGG and one out of ten subjects responded to BPO.BGG (Table 2).

Lymphocyte transformation test

The relatively low concentrations of CEPR used in this study were chosen because higher concentrations produced an inhibition of transformation. This effect will be

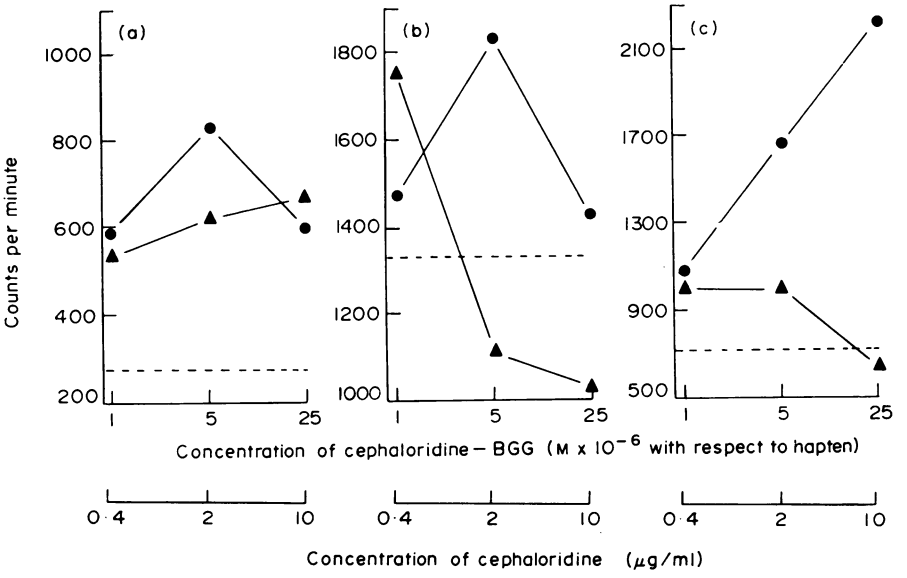


FIG. 3. Lymphocyte transformation (stimulation) test in three penicillin-allergic patients, carried out in presence of cephaloridine (▲) and in the presence of cephaloridine–bovine gamma-globulin conjugate (●). The response is measured by the incorporation of [^{3}H]thymidine. (– – –) Control.

discussed in a separate paper (Assem and Vickers, in preparation). In the penicillin-allergic group positive lymphocyte transformation was obtained in ten out of twenty patients (50 per cent) with CEPR.BGG and eight out of twenty (40 per cent) with CEPR (Table 2). Some typical dose–response curves are shown in Fig. 3. Three patients gave positive responses to CEPR.BGG in the absence of a response to BPO.BGG or to B.Pen. Only one of these had received ceporin. On the other hand, in seven patients a lymphocyte

TABLE 3

RESULTS OF INVESTIGATION IN A PENICILLIN-ALLERGIC PATIENT WHO HAD NOT RECEIVED CEPORIN

Test system	Benzylpenicillin		Benzylpenicilloyl conjugates			Cephaloridine		Cephaloridine conjugates		
	2×10^{-4}M	2×10^{-3}M	1×10^{-5}M	5×10^{-5}M	25×10^{-5}M	2×10^{-4}M	1×10^{-3}M	1×10^{-5}M	5×10^{-5}M	25×10^{-5}M
Intradermal skin test										
Diameter of wheal(mm)	—	7	—	<5	8	7	7	6	7	8
Diameter of flare (mm)	6	10	6	8	20	18	16	10	17	35

Test system	Control	Benzylpenicillin	Benzylpenicilloyl conjugates			Control	Cephaloridine			Cephaloridine conjugates		
		2×10^{-4}M	1×10^{-5}M	5×10^{-5}M	25×10^{-5}M		4×10^{-5}M	2×10^{-4}M	1×10^{-3}M	1×10^{-5}M	5×10^{-5}M	25×10^{-5}M
Leucocyte histamine release												
Percentage of total histamine	2·2	1·9	8·1	11·8	7·3	5·0	8·9	10·3	12·1	15·8	19·7	27·4

Test system	Control	Benzylpenicillin		Benzylpenicilloyl conjugates			Control	Cephaloridine			Cephaloridine conjugates		
		2×10^{-6}M	2×10^{-5}M	1×10^{-6}M	5×10^{-6}M	25×10^{-6}M		4×10^{-7}M	2×10^{-6}M	1×10^{-5}M	1×10^{-6}M	5×10^{-6}M	25×10^{-6}M
Lymphocyte transformation test													
Mean cpm ±s.d.	267± 35·6	369± 71·3	371·0± 61·1	1321·7± 349·5	842·5± 106·5	1652·2± 50·6	235·6± 44·3	208·2± 21·4	221·9± 10·6	114·7± 22·9	245·7± 10·0	247·0± 10·2	209·4± 25·0

The concentration of the conjugates is shown as the molarity of the haptenic group.

Note that the skin tests, and the leucocyte histamine release tests are positive both with cephaloridine and its conjugates. On the other hand, the response to benzylpenicillin is weak in the skin test and negative in the leucocyte histamine release test. The response to benzylpenicilloyl conjugates in these two tests is also weaker than the response to cephaloridine conjugates. It was of interest, however, that penicilloyl conjugates produced a strongly positive lymphocyte transformation test, while the test with cephaloridine conjugates was negative.

response was obtained with BPO.BGG but not with CEPR.BGG. Seven patients responded to both BPO.BGG and CEPR.BGG. Thus a response to BPO.BGG and/or CEPR.BGG was obtained in seventeen out of twenty cases (85 per cent). In the control group no positive reactions to CEPR.BGG were obtained. An example of the results of various tests in a penicillin-allergic patient is shown in Table 3.

DISCUSSION

Haemagglutinating antibodies

Serum haemagglutinating antibodies which react with CEPR were present in a large number of patients who had not received cephalosporin therapy, irrespective of whether they were allergic to penicillin or not. Similar proportions of penicillin-allergic (thirty-six out of thirty-nine) and non-penicillin-allergic (thirty out of thirty-five) patients gave positive responses, indicating that the antibodies detected are non-sensitizing. No quantitative conclusions can be reached from these results since the difference in titre may merely represent differences in the SRBC preparations with CEPR.HSA and with BPO.HSA. However, the CEPR titres were generally below the BPO titres, a four-fold difference being the most common. The common occurrence of a fixed ratio suggests cross-reaction. Against the latter explanation is the fact that some patients who had not received cephalosporins had higher or equal titres with CEPR.HSA-coated red cells (Table 1). This finding by itself suggests that, apart from cross-antigenicity between penicillins and cephalosporins, there is another possibility, namely the occurrence in a proportion of subjects, if not in all, of cephalosporin antibodies due to natural exposure to cephalosporin C. The same finding strengthens the argument in favour of the immunological response to natural exposure to cephalosporins put forward by Abraham, Petz and Fudenberg (1968). The latter authors' argument was entirely based on the detection of cephalothin antibodies in control subjects in their series who had not received any cephalosporin derivatives. Although the cephaloridine antibodies detected by the haemagglutination technique in thirty-one out of thirty-five of our normal control subjects who had not received cephalosporins are of the non-tissue sensitizing type, it is possible that natural exposure to cephalosporins could initiate the production of sensitizing antibodies, and hence, may explain the occurrence of an allergic response on a first therapeutic exposure. An example of this situation has been reported by Kaplan and Weinstein (1967).

When BPO-specific antibodies were removed by absorption the ability of the serum to agglutinate CEPR.HSA-coated SRBC was diminished or completely abolished. It seems, therefore, that cross-reaction of CEPR with penicillin is largely due to reaction with the BPO group. The splitting of the β-lactam ring in cephalosporins would give a cephalosporoyl group which, when conjugated to protein, would possess structural areas in common with the BPO conjugate. These findings are in agreement with earlier work by Ky *et al.* (1970) and Feinberg (1968), but in conflict with the suggestion by Newton and Hamilton-Miller (1967) that a 'cephalosporoyl' derivative would be unstable.

In serum from seven out of eighteen patients (of these seven only one had received CEPR) absorption studies with BPO.HSA-coated red cells showed incomplete removal of antibodies capable of agglutinating CEPR.HSA-coated SRBC, suggesting high specificity of the residual antibodies, which may lend further support to the suggestion of previous exposure (through natural routes) to compounds related to CEPR.

Skin tests

The skin reactions observed are similar to those reported by Girard (1968) and contradict the earlier observations of Stewart (1962) who reported no cross-allergenicity of cephalosporin C with penicillins in skin tests. However, he used the unconjugated drug only. The responses to CEPR in the absence of responses to CEPR.HSA in our series may indicate specificities other than the cephalosporoyl determinant.

Nine patients responded to CEPR in the absence of reaction to B.Pen. One of these had received CEPR and developed an allergic reaction. Her response may, therefore, represent specificity for determinants which do not cross-react with penicillin. In the other eight, a sub-clinical sensitization to cephalosporins from natural sources is possible, or their sensitization may be due to cross-reaction with antibodies against the minor penicillin determinants. However, in five of these patients, skin tests with benzylpenicillenate–HSA and benzylpenicillamine–HSA (the two main minor determinants) were negative. However, in view of the unreliability of skin testing no conclusion can be drawn from these results. Four patients reacted to B.Pen but not to CEPR. This is probably due to allergy to minor penicillin determinants which do not cross-react with corresponding breakdown products of CEPR. Six patients reacted to BPO.HSA but not to CEPR.HSA. Three of these had a positive leucocyte test with CEPR.HSA, thus suggesting a false negative skin test, while the other three were negative in the latter test, suggesting absence of cross-allergenicity.

Leucocyte challenge test

In this test the use of hapten–protein conjugates with appropriate carriers is necessary to achieve a sensitive and reliable system (Vickers and Assem, 1974). With CEPR.BGG positive results were obtained in 80% of penicillin-allergic patients, whereas none of the ten controls reacted, excluding a non-specific response.

Three patients responded to CEPR.BGG in the absence of response to BPO.BGG or B.Pen and a further five gave a greater response to CEPR.BGG than to BPO.BGG. Only two of these patients had previously received CEPR, and it is possible that they have reaginic antibodies which do not cross-react with penicillin. In the six other patients sub-clinical sensitization to CEPR from natural sources (e.g. exposure to cephalosporin C) is possible but unlikely to be the whole explanation, considering the extent of the reactions. Cross-reaction with antibodies of minor penicillin determinant specificity is also possible. However, there is evidence (Assem and Vickers, in preparation) that cephaloridine can potentiate the action of other allergens in this system, and thus it is plausible to suggest that a self-potentiation reaction has occurred. Alternatively, CEPR.BGG may either bind more effectively (possibly due to greater affinity) to penicillin-specific antibodies or may induce greater mediator release than BPO.BGG because of a more effective triggering mechanism induced through the commonly accepted configurational change in the Fc portion of the IgE molecule.

The lymphocyte transformation test

The use of hapten–protein conjugates is of relatively little use in this test as compared with the leucocyte challenge (Assem and Vickers, 1972; Vickers and Assem, 1974). In contrast with the high response in the leucocyte challenge test, the number of patients giving positive LTT to CEPR (eight out of twenty) and CEPR.BGG (ten out of twenty) is surprisingly low since the LTT measures both immediate and delayed reactions. No

positive results were obtained in the control group, again excluding a non-specific response in allergic patients.

In view of the inhibition of transformation seen in control subjects with CEPR and in penicillin-allergic patients even with low CEPR concentrations and with CEPR.BGG, the results of the LTT are difficult to assess. The low and negative responses may represent a combination of effects, inhibition of DNA synthesis, assumed from the reduced incorporation of [^{3}H]thymidine, plus immunological stimulation of transformation.

In common with previous studies, including that by Ky *et al.* (1970), the number of patients tested may be too small to allow any estimate to be made of the number of penicillin-sensitive subjects likely to react to cephalosporin therapy. However, our study differs in that we have used four test systems and have tested not only with CEPR but also with protein conjugates of this drug. In addition we have used various concentrations in order to obtain dose–response curves with both allergen preparations. Our studies have indicated the critical value of allergen concentrations: CEPR and also CEPT (Assem and Vickers, in preparation) may inhibit lymphocyte transformation in concentrations of the order of 10 μg/ml. The relatively low figure for positive LTT and CEPR obtained by Ky *et al.* (1970) (12 per cent compared with 40 per cent in our series) in penicillin-allergic patients may thus have been due to the use of a CEPR concentration (16·6 μg/ml) which can inhibit lymphocyte transformation. The difference between our results and those of Ky *et al.* (1970) cannot be due to selection of patients, since in contrast with our figure of 40 per cent for a positive LTT with B.Pen their results showed 100 per cent positive. The differences in technique used to estimate transformation may account for the discrepancies in the two studies. We measured DNA synthesis through the estimation of [^{3}H]thymidine incorporation by lymphocytes, whereas Ky *et al.* (1970) used morphological examination to determine transformation. This latter technique may give a high percentage of false positive results.

Tests in subjects who are not allergic to penicillin were negative in both series, thus excluding non-specific responses.

We are the first to use the leucocyte challenge tests in studies of cross-reaction between cephalosporins and penicillin. This test, which is a correlate of immediate-type allergy, and is thus of great value in detecting such a potentially serious reaction, has given us the highest figure for allergy to CEPR in penicillin-allergic patients (80 per cent).

In the discussion of the paper by Ky *et al.* (1970), Dr R. D. Foord of Glaxo Laboratories reported a clinical incidence of allergy to cephaloridine of 1 and 10 per cent in normal and penicillin-allergic patients respectively; Dash, Foord, Johnson and Cooper (1972) reported an incidence of allergy to cephalexin of 1·1 and 8·2 per cent respectively. Figures around 20 per cent have been reported by other workers (a small series by Welch (1966), by Abraham *et al.* (1968) and by Thoburn, Johnson and Cluff (1966)). The highest clinical incidence so far reported is that by Molthan (1968). In a series of twenty-five penicillin-allergic patients treated with cephalothin, one had severe anaphylactic shock, thirteen had haemolytic anaemia, and others had severe rashes, drug fever and serum sickness. In our series, three of the penicillin-allergic patients received cephaloridine, and all developed severe allergic reactions. From our *in vitro* tests it seems probable that others would develop reactions if given cephaloridine.

The failure of other groups to observe allergy to cephalosporins clinically in as high a proportion of penicillin-allergic patients as we have observed in our series may be due to one or more of the following reasons. (1) Our patients are well documented cases of

penicillin allergy, while in other series the investigations carried out have usually been incomplete, and thus the conclusions drawn may have been wrong. (2) Allergy tests or clinical response to cephalosporin therapy in other series may not have taken place at the time of optimal response, i.e. occurred too soon or too long after an allergic reaction to penicillins (Welch (1966), extrapolating from studies in penicillin-allergic patients by Budd, Parker and Norden (1964)). This seems unlikely since we have obtained evidence of allergy to penicillins and cephaloridine 20 years after a single course of benzylpenicillin only (patient number 8). (3) It has been suggested that cross-antigenicity does not necessarily imply cross-allergenicity (*Lancet*, 1967; Stewart, 1967). Although it is possible it seems rather unlikely. Our results do not support this view. (4) The rather special effects of cephalosporins in the LTT, which are possibly due to inhibition of DNA synthesis, may 'damp down' allergic reactions.

Despite all these possibilities, which only tend to underestimate or reduce the incidence of cephalosporin allergy, the greater frequency of cephalosporin allergy in penicillin-allergic patients has never been disputed, and the manufacturers recognize this risk (Foord, 1970; Dash *et al.*, 1972), but they point out that it is difficult to guess the likely incidence of true cross-reactivity compared with a primary cephalosporin allergy developing in a penicillin-allergic individual. We can, perhaps, draw from our studies the following important conclusions. (1) Both cephalosporins and penicillins have cross-reacting major antigenic determinants, the cephalosporyl and penicilloyl groups respectively. (2) Both this cross-reaction and the natural exposure to cephalosporins seem to play some part in allergy to cephalosporins. (3) Minor antigenic determinants of penicillins and cephalosporins do not cross-react as readily as the major determinants. (4) Since cross-allergenicity between cephaloridine and benzylpenicillins, which have different side chains, was elicited by the leucocyte histamine release test in 80 per cent of the penicillin-allergic patients, it would appear that the side chain plays a minor role in cross-allergenicity.

Although the information cards of pharmaceutical firms mention penicillin allergy as a contra-indication for cephalosporin therapy, or at least point out the caution needed under these circumstances, commercial advertisements and even some medical journals (e.g. Prescribers' Journal, December 1971) may suggest that in penicillin-allergic patients cephalosporins are safe alternatives. It is important to distinguish between the relative risk of cephalosporin therapy in penicillin-allergic patients in conditions of serious infections (e.g. infective endocarditis), where penicillin is probably the most effective treatment, and in cases where such therapy is only given as a prophylactic cover. In the former situation the infection itself may be more serious than a potential reaction to cephalosporins. In these circumstances the best approach would be to hyposensitize the patient to penicillin, either by using the drug by itself or with a monovalent hapten, while maintaining proper cover with anti-allergic drugs. Alternatively the same procedure could be followed, but using cephalosporins in place of penicillin. This may reduce the potential risk of reaction, but the effectiveness of antibiotic therapy may also be reduced. In hospitals where hyposensitization procedures are not practised, then cephalosporins could be used, but with extreme caution.

Where prophylactic antibiotic therapy is required, e.g. when undergoing dental surgery, the risk of reaction to cephalosporins in penicillin-allergic patients may be too great to take. In patients not predisposed to injections an alternative antibiotic should always be given, and even in patients who are prone to infective endocarditis the risk of cephalosporin therapy may be relatively greater than the risk of infection. The Cardiac

Society has recently been engaged in reviewing this problem (H. A. Fleming, personal communication).

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