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IMMUNOHAEMATOLOGICAL CROSS-ALLERGENICITY BETWEEN PENICILLIN AND CEPHALOTHIN IN HUMANS

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SUMMARY

The immunogenicity of cephalothin and its cross-reactivity with penicillin were studied in a series of 174 adult patients receiving intravenous sodium cephalothin or intravenous penicillin-G.

Immunogenicity was indicated by a higher incidence of anti-cephalothin antibody in patients after having received the drug than in controls and by an increase in titre of anti-cephalothin antibody during administration of the drug. Cross-allergenicity was indicated by a high incidence of anti-cephalothin antibodies in patients receiving penicillin who had never received cephalothin and by an increase in anti-cephalothin antibody titre in patients receiving penicillin. Further, patients receiving cephalothin had a high incidence of anti-penicillin antibodies and the anti-penicillin antibody titre increased during cephalothin administration. Confirmation of cross-allergenicity was obtained by inhibition of haemagglutination by penicillin and cephalothin derivatives as well as by absorption of sera with erythrocytes sensitized with penicillin or cephalothin. Treatment of sera with 2-mercaptoethanol indicated that anti-cephalothin and anti-penicillin antibodies were largely of the IgM immunoglobulin class. A low incidence of positive direct Coombs tests was found in this series and the probable reasons for this are discussed.

Penicillin is the most common cause of drug-immune haemolysis for which serological evidence of a drug related antibody exists. Since there is clear immunohaematological evidence of cross-allergenicity between penicillin and cephalothin, it is surprising that haemolytic anaemia due to cephalothin administration has not as yet been reported. This may be due to the fact that neither cephalothin alone nor a cephalothin–anti-cephalothin–antibody complex are firmly bound to erythrocytes during therapeutic administration of the drug.

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INTRODUCTION

Cephalothin, a semi-synthetic derivative of 7-aminocephalosporanic acid, is a broad spectrum antibiotic which is structurely related to the penicillins. The cephalosporins differ from the penicillins in that a six-membered dihydrothiazine ring replaces the five-membered thiazolidine ring of the parent compound. The extent of immunological cross-reactivity between cephalothin and penicillin is an important clinical problem which concerns not only the usual drug allergic manifestations but also immunohaematological abnormalities such as positive direct Coombs tests caused by cephalothin (Gralnick, Wright & McGinniss, 1967; Molthan, Reidenberg & Eichman, 1967; Perkins, Saslaw & Billmaier, 1967) and immunohaemolytic anaemia caused by penicillin (Petz & Fudenberg, 1966; Swanson, Chanmougan & Schwartz, 1966). Experiments in animals and clinical experiences have led to inconclusive findings concerning the extent of cross-allergenicity in humans.

The present study was designed to characterize the immune response to cephalothin and the extent of cross-allergenicity with penicillin in humans by a variety of immuno-haematological techniques.

MATERIALS AND METHODS

Patients studied

A total of 174 consecutive adult patients from the medical surgical, and surgical specialty wards of the San Francisco General, University of California, United States Veteran Administration, Southern Pacific Memorial, and Letterman General Hospitals who were receiving intravenous sodium cephalothin or intravenous aqueous penicillin-G were studied. Of these, 125 patients received penicillin and forty-nine received sodium cephalothin.

The patients were divided into two groups for the purposes of analysis. Group 1 consisted of those patients from whom only one blood sample was available and was further subdivided into groups 1-Pen and 1-Ceph consisting of those patients who received penicillin or cephalothin, respectively. Group 2 consisted of patients from whom serial blood samples were available and was similarly subdivided. All patients in both groups had received penicillin in the past. The dosage range of penicillin varied from 2·0 to 100 million units/day, with 79/125 (62%) patients receiving 10–20 million units/day; that of cephalothin was 1·0–12·0 g/day, with 33/49 (67%) patients receiving from 4 to 6 g/day. Nine patients who received cephalothin recalled a history of allergy to penicillin.

Sera were also obtained from thirty-nine normal hospital personnel who had received penicillin but not cephalothin in the past.

Preparation of hyperimmune rabbit sera

Rabbit antisera to cephalothin and benzylpenicillin were prepared in New Zealand white rabbits by intradermal injection of an emulsion of penicillin or cephalothin in an equal volume of complete Freund's adjuvant at multiple sites. Each dose contained 1·0 million units of benzylpenicillin-G or 250 mg of cephalothin, either as an aqueous solution or bound to autologous serum protein (prepared by incubation at 37°C for 3 hr).

The animals were immunized weekly for 4–6 weeks followed by rest periods of several months for a total period of time of up to 1 year. The sera thus obtained were utilized in

preliminary experiments to standardize the haemagglutination techniques for detection of anti-penicillin and anti-cephalothin antibodies and as positive controls in subsequent experiments. Pre-immunization sera were used for negative controls.

Serological tests

Cephalothin sensitized erythrocytes were prepared by adding cephalothin (to a concentration of 10 mg/ml) to a 5% suspension of washed group O-positive erythrocytes in phosphate buffered saline (pH 7·4). The mixture was incubated for 1 hr at 37°C with gentle mixing every 15 min. The sensitized cells were washed and stored at 4°C in a solution prepared by the method of Levine, Fellner & Levytska (1966a) for no longer than 1 week before use in haemagglutination and indirect Coombs tests.

Cells were sensitized with penicillin by the method of Levine *et al.* (1966a). Fresh O-positive erythrocytes were washed three times in normal saline and 1 ml of the washed packed cells added to a solution of 0·6 g of benzylpenicillin-G (about 1000000 units) dissolved in 20 ml of trimethylamine hydrochloride (pH 10·0); the mixture was incubated for 60 min at 25°C with mixing every 15 min.

Haemagglutination titrations were performed using dextran–normal rabbit serum–Tris buffered saline (Dex–NRS–TBS) diluent as described in detail by Levine *et al.* (1966a). With hyper-immune anti-cephalothin rabbit antiserum (absorbed to remove all anti-penicillin antibody) and cephalothin-sensitized cells prepared as described above, preliminary testing of other diluents (phosphate buffered saline, pH 7·4; pooled whole human serum) showed the Dex–NRS–TBS diluent to yield the highest haemagglutination titres.

For indirect Coombs tests and absorption tests, penicillin sensitized red cells were prepared as described for cephalothin and, in addition, were incubated at 4°C for 16 hr before placing in the storage solution. The concentration of penicillin in the red cell suspension was 20000 units/ml.

Absorption tests with antibiotic sensitized erythrocytes were performed by incubating equal volumes of packed erythrocytes and the patient's serum for 60 min at room temperature; after centrifugation, the supernatant was removed and tested by haemagglutination. As a control, the patient's serum was incubated with unsensitized red cells from the same donor, and the supernatant tested.

Inhibition tests were performed in a checkerboard fashion with mixtures of equal volumes of serial dilution of both the patient's serum and the potential inhibitors dissolved in Dex–NRS–TBS diluent (Levine *et al.*, 1966a). The combinations were incubated for 60 min at room temperatures, cephalothin or penicillin sensitized cells were added, and the haemagglutination titre determined after a further 45-min incubation. Appropriate cell controls were run, and the specificity of inhibition tested by failure of the inhibitors to inhibit unrelated anti-Rh_0 agglutination.

Indirect Coombs tests were performed by adding a 2% suspension of cephalothin or penicillin sensitized, or unsensitized control cells to equal volumes of serial dilutions of the patient's serum in normal saline (pH 7·4). The cells were incubated at 37°C for 1 hr, and washed four times in normal saline. Antiglobulin reagent was then added, the mixture centrifuged for 15 sec in a Serofuge (Clay–Adams), and agglutination scored 0 to + + + +; the titres were expressed as the highest serum dilution giving a positive reaction.

The direct Coombs test was performed by adding commercial broad spectrum antiglobulin reagent or a broad spectrum antiglobulin serum prepared in our laboratory to an

equal volume of a 2% suspension of the patient's washed erythrocytes. This mixture was spun for 15 sec in a Serofuge and agglutination graded 0 to ++++.

Tests for in vivo sensitization of erythrocytes by antibiotic

Erythrocytes from sixty-six patients were tested with rabbit anti-penicillin and anti-cephalothin sera in an attempt to demonstrate whether coating of erythrocytes with cephalothin or penicillin can occur *in vivo*.

Freshly drawn, saline suspended red cells were washed three times in Tris-buffered saline (pH 7·4), and a 2% suspension of the cells prepared. One drop of these cells was added to a 1:8 dilution of one or more immune rabbit serums in Dex–NRS–TBS diluent, incubated at room temperature for 1 hr, spun for 1 min in a serofuge, read and agglutination graded from 0 to ++++. Appropriate positive and negative cell, diluent, and serum controls were concomitantly tested. Rabbits numbers 21 and 22 were immunized with penicillin; rabbit number 28 was immunized with cephalothin. Titres of these sera against erythrocytes optimally sensitized *in vitro* were also determined.

Mercaptoethanol sensitivity

Mercaptoethanol tests were performed as previously described (Fudenberg, Kunkel & Franklin, 1959). One-tenth volume of 1 M 2-mercaptoethanolamine hydrochloride in buffered saline (pH 7·4) was added to 1 volume of the patient's serum of highest titre and this mixture was incubated at 37°C for 1 hr. The serum was then tested by haemagglutination titration without dialysis. In parallel controls, performed simultaneously, no change occurred in titre of an IgG anti-Rh_0 antibody, and there was a reduction in titre of an IgM cold agglutinin from greater than 1:5000 to 1:4.

RESULTS

Sera from thirty-nine normal persons were titrated against penicillin and cephalothin sensitized erythrocytes; the results are illustrated in Table 1. Although all of these persons

TABLE 1. Distribution of haemagglutination titres in thirty-nine normal persons using penicillin and cephalothin sensitized erythrocytes

Reciprocal of haemagglutination titre	No. of persons giving haemagglutination reactions	
	Penicillin sensitized erythrocytes	Cephalothin sensitized erythrocytes
<4	9 (22·5%)	12 (31·0%)
4	14 (38·0%)	6 (15·0%)
8	9 (22·5%)	9 (22·5%)
16	2 (5·0%)	4 (10·0%)
32	4 (10·0%)	5 (12·5%)
64	1 (2·5%)	2 (5·0%)
128	0	1 (2·5%)

had received penicillin in the past, none had received penicillin during the last 2 years and none had ever received cephalothin.

TABLE 2. Group 1-Pen: distribution of haemagglutination titres and duration of therapy of ninety-three patients receiving penicillin-G from whom a single serum specimen was obtained

	Reciprocal of haemagglutination titre	No. of persons giving positive haemagglutination titres			
		Less than 3*	3–7*	8 or more*	Totals
Penicillin sensitized erythrocytes	8	1	0	0	1
	16	6	6	0	12
	32	6	0	1	7
	64	3	6	1	10
	128	0	1	3	4
	256	0	2	0	2
	512	1	2	0	3
	1000	0	2	1	3
	2000	1	0	0	1
		18/46 (39%)	19/35 (57%)	6/12 (50%)	43/93 (46%)
Cephalothin sensitized erythrocytes	8	1	6	0	7
	16	4	3	0	7
	32	2	2	2	6
	64	1	2	0	3
	128	1	0	0	1
	256	1	0	0	1
		10/46 (22%)	13/35 (37%)	2/12 (17%)	25/93 (27%)

*Duration of therapy (days).

TABLE 3. Group 1-Ceph: distribution of haemagglutination titres and duration of therapy of thirty-three patients receiving cephalothin from whom a single serum specimen was obtained

	Reciprocal of haemagglutination titre	No. of persons giving positive haemagglutination titres			
		Less than 3*	3–7*	8 or more*	Totals
Cephalothin sensitized RBC	8	5	2	0	7
	16	0	2	0	2
	32	0	3	0	3
	64	0	0	0	0
		5/16 (31%)	7/16 (44%)	0/1 (0%)	12/33 (36%)
Penicillin sensitized RBC	8	3	1	0	4
	16	1	2	0	3
	32	0	0	0	0
	64	1	1	0	2
	128	0	1	0	1
	256	0	0	0	0
	512	0	1	0	1
		5/16 (31%)	6/16 (37%)	0/1 (0%)	11/33 (33%)

* Duration of therapy (days).

Group 1-*Pen*

Single serum samples of ninety-three patients who received penicillin from 1 to 20 days were tested (Table 2). Dosages ranged from 2·0 to 80 million units of penicillin/day; sixty-six of ninety-three (71%) received 10–30 million units/day. Considering haemagglutination titres of 1:8 or greater, forty-three of the ninety-three sera (46%) gave positive results using penicillin coated erythrocytes, and nineteen of ninety-three (20%) using both penicillin and cephalothin-coated cells. No correlation was noted between the dose of penicillin and the height of the titre.

Eighteen of forty-six (39%) patients who received penicillin for less than 3 days had titres of 1:8 or greater, and thirteen out of eighteen (73%) of these titres were 1:32 or less. Nineteen of thirty-five (57%) patients on penicillin for 3–7 days and six out of twelve (50%) of those patients on penicillin for more than 8 days, had haemagglutination titres with penicillin coated erythrocytes of 1:8 or greater. The median positive titre varied according to duration of penicillin therapy, and was 1:32 for the 'less than 3 day' group, 1:64 for the '3–7 day' group, and 1:128 for the '8 days or more' treatment group.

Using cephalothin sensitized erythrocytes, the number of positive haemagglutination titres for each time-duration treatment group was ten out of forty-six (22%), thirteen out of thirty-five (37%) and two out of twelve (17%), respectively; no differences were noted in the median positive haemagglutination titre.

Group 1-*Ceph*

Similarly, single serum samples from thirty-three patients who received cephalothin from 1 to 16 days were tested (Table 3). Twelve of thirty-three (36%) had haemagglutination titres of 1:8 or greater with cephalothin coated red cells, and eleven out of thirty-three (33%) with penicillin-sensitized erythrocytes; six samples (18%) were positive with both penicillin and cephalothin-coated erythrocytes. The dosage range varied from 1·0 to 12·0 g/24 hr. Six patients had a history of penicillin allergy; three of the patients were vague as to the nature of the reaction, and other reactions consisted of skin eruptions. No allergic reactions developed in any of these patients while receiving cephalothin.

Of the patients who received cephalothin for less than 3 days, five out of sixteen (31%) had positive haemagglutination titres with cephalothin and with penicillin sensitized erythrocytes. Further, of those patients who received cephalothin from 3 to 7 days, seven out of sixteen (44%) showed positive titres with cephalothin coated red cells, and six out of sixteen (37%) with penicillin coated cells. Only one patient received cephalothin for greater than 8 days and both haemagglutination titres were negative.

Group 2

Thirty-two patients who received intravenous penicillin (Group 2-Pen), and sixteen who received cephalothin (Group 2-Ceph), were followed with weekly blood samples during their hospitalization to note development of, or change in, haemagglutination titres using penicillin and cephalothin coated cells (Table 4). Dosage range for penicillin was 6·0–100 million units/day and that of cephalothin 2–12·0 g/day. A rise in titre was defined as a two tube or greater increase using doubling dilutions of the patient's serum.

Group 2-*Pen*

The duration of penicillin therapy prior to obtaining the final sample ranged from 2 to 39 days with a median of 9 days. Twenty-nine of thirty-two (91%) had a haemagglutination

titre of 1:8 or greater at some time during the course of therapy and twenty out of thirty-two (63%) a rise in haemagglutination titre with penicillin sensitized red cells. Further, using cephalothin sensitized erythrocytes, twenty-two out of thirty-two (69%) showed a titre of 1:8 or greater in at least one sample, and six out of thirty-two (19%) an increase in titre. One patient developed a titre of 1:16 against cephalothin-sensitized erythrocytes but his titre against penicillin sensitized erythrocytes was negative (less than 1:8).

Of the twenty patients demonstrating an increase in antipenicillin antibody titre, twelve out of eighteen (67%) developed the rise during the 1st week of therapy, four out of eighteen (22%) during the 2nd week, and two out of eighteen (11%) during the 3rd week. (Two patients were not included here because of a discontinuity in the sampling.)

TABLE 4. Group 2: patients receiving penicillin-G or cephalothin from whom weekly serial samples were obtained

		Patients who received penicillin Group 2-Pen		Patients who received cephalothin Group 2-Ceph	
		Penicillin sensitized erythrocytes	Cephalothin sensitized erythrocytes	Penicillin sensitized erythrocytes	Cephalothin sensitized erythrocytes
Patients with positive haemagglutination titres of 1:8 or greater in at least 1 serial sample		29/32 (91%)	22/32 (69%)	13/16 (81%)	12/16 (75%)
Patients with increase in titre of 2 tubes or greater		20/32 (63%)	6/32 (19%)	4/16 (25%)	4/16 (25%)
Week of therapy during which titre increased	1	12/18 (67%)	4/6 (66·7%)	1/4 (25%)	1/4 (25%)
	2	4/18 (22%)	1/6 (16·6%)	2/4 (50%)	3/4 (75%)
	3	2/18 (11%)	1/6 (16·6%)	1/4 (25%)	0

Of the six patients demonstrating rises in titre with cephalothin coated cells, four developed the increase during the 1st week of therapy and also showed increases in penicillin antibody titre. One of the remaining two increased during the 2nd week of penicillin therapy and the titre was less than 1:8 with penicillin coated cells. The other increased during the 3rd week of therapy, 7 days after a rise in penicillin titre.

Group 2-Ceph

The duration of cephalothin therapy prior to obtaining the final sample ranged from 4 to 25 days with a median of 10 days. Thirteen of sixteen (81%) had a haemagglutination titre of 1:8 or greater with penicillin coated erythrocytes and twelve out of sixteen (75%) a titre of 1:8 or greater with cephalothin coated cells in at least one of the samples tested.

Four of sixteen (25%) demonstrated an increase in titre using cephalothin coated red cells. One patient demonstrated an increase in titre during the 1st week of therapy, and the remaining three (75%) increased during the 2nd week of treatment. Two patients showed a concomitant increase in penicillin haemagglutination titre.

In this group, three out of sixteen patients had a previous history of penicillin allergy, and one of these developed a skin reaction while receiving cephalothin.

Four of sixteen (25%) showed a rise in titre with penicillin sensitized cells (in one of these, the titre using cephalothin sensitized erythrocytes was less than 1:8), one showing the increase in titre during the 1st week of therapy, two during the 2nd week, and one during the 3rd week.

No correlation was noted in either group between the dose of the drug given and increases in, or height of titre.

TABLE 5. Haemagglutination of *in vitro* sensitized erythrocytes and erythrocytes from patients receiving penicillin or cephalothin by sera from rabbits immunized with penicillin or cephalothin

	Erythrocytes used	Rabbit No. 21 Pen	Rabbit No. 22 Pen	Rabbit No. 28 Ceph
Haemagglutination titre of rabbit sera using *in vitro* sensitized erythrocytes	Penicillin sensitized RBC	1:512	1:4000	1:256
	Cephalothin sensitized RBC	1:512	1:512	1:64000
Percentage of positive haemagglutination reactions using rabbit sera and patient's erythrocytes	Erythrocytes from patients receiving cephalothin	0/15	2/12 (16·6%)	0/10
	Erythrocytes from patients receiving penicillin	0/17	14/12 (64%)	0/7

Direct Coombs tests

Two hundred and forty-eight direct Coombs tests were performed on the 174 patients. Only three out of 125 (2·4%) patients on intravenous penicillin and one out of forty-nine (2%) patients on intravenous cephalothin developed a positive direct Coombs test.

In vivo coating of patient erythrocytes during treatment

An attempt to demonstrate the *in vivo* coating of erythrocytes by penicillin or cephalothin was performed using rabbit antisera to penicillin and cephalothin. The rabbit sera used contained the haemagglutination titres toward erythrocytes optimally sensitized *in vitro* with penicillin and cephalothin as noted in Table 5.

Freshly drawn erythrocytes from sixty-three patients who were receiving either of the two drugs were tested with one or more rabbit sera. Sera from rabbits 21 and 28 failed to give positive reactions. With serum from rabbit 22, twelve out of twenty-two (64%) patients receiving penicillin, and two out of twelve (16·7%) patients receiving cephalothin, gave positive reactions. All patients receiving penicillin who gave positive reactions had received doses of 10 million units/day or greater, and all but one of the patients had been treated for at least 3 days. However, some patients who received similar doses for comparable periods of time gave negative reactions.

The erythrocytes of one patient receiving penicillin who had a positive direct Coombs

TABLE 6. Molar concentration of drug required for inhibition of haemagglutination

Patient	Patient's haemagglutination titre *vs.* penicillin or cephalothin RBC		Sensitized erythrocytes utilized in inhibition tests	Molar concentration of drug causing inhibition			
	Pen	Ceph		Penicillin-G	Benzylpenicilloyl polylysine	Cephalothin	Cephaloridine
P.H.	1:1000	Neg	Penicillin	$1{\cdot}5\times10^{-2}$	4×10^{-6}	NI	NI
H.A.	1:2000	1:16	Penicillin	12×10^{-2}	6×10^{-5}	NI	NI
J.P.	1:128	Neg	Penicillin	2×10^{-3}	4×10^{-6}	PI $1{\cdot}25\times10^{-3}$	NI
G.P.	1:256	Neg	Penicillin	$7{\cdot}5\times10^{-3}$	$1{\cdot}5\times10^{-5}$	PI 3×10^{-3}	PI 5×10^{-3}
K.M.	1:128	1:256	Cephalothin	3×10^{-2}	$1{\cdot}2\times10^{-4}$	$1{\cdot}5\times10^{-4}$	NI
P.G.	1:32	1:512	Cephalothin	4×10^{-3}	PI 6×10^{-5}	4×10^{-4}	$1{\cdot}25\times10^{-3}$
I.M.	1:16	1:128	Cephalothin	NI	NI	PI $2{\cdot}0\times10^{-4}$	PI $1{\cdot}25\times10^{-3}$

NI, Non-inhibitory to concentration of 4×10^{-2} M/L penicillin-G, 5×10^{-5} M/L benzylpenicilloyl polylysine, 6×10^{-3} M/L cephalothin, and 5×10^{-3} M/L cephaloridine.

PI, Partial inhibition: first noted at concentration indicated; total inhibition not achieved.

test was tested with serum of rabbit 22. This anti-penicillin serum caused + + + + agglutination thus indicating serologically that the erythrocytes were coated with both penicillin and antibody.

The two patients who received cephalothin and gave positive reactions received 4·0 g for 16 days and 6·0 g for 17 days, and both had mildly elevated blood urea nitrogens.

Inhibition of haemagglutination

Inhibition studies were performed with seven sera which contained penicillin or cephalothin antibody or both in high titre, in order to ascertain the degree of inhibition, and cross-reactivity of the respective antibody with penicillin, benzylpenicilloyl polylysine, cephalothin and cephaloridine. The results are noted in Table 6.

All four anti-penicillin antibodies and two of the three anti-cephalothin antibodies were inhibited by penicillin derivatives. Inhibition of anti-cephalothin antibody by cephalosporin C derivatives varied from complete inhibition to total lack of inhibition. There were two instances of partial inhibition of anti-penicillin antibody by cephalosporin C derivatives.

Absorption studies

A total of forty-eight sera were absorbed with both penicillin and cephalothin-sensitized erythrocytes. Of these sera, forty-four had an anti-penicillin antibody titre of 1:8 or greater, thirty-four had an anti-cephalothin titre of 1:8 or greater and twenty had an anti-cephalothin titre of 1:16 or greater. Absorption with cephalothin sensitized erythrocytes removed all penicillin and cephalothin haemagglutinating activity in one or two absorptions. Absorbing with penicillin sensitized erythrocytes resulted in removal of anti-penicillin antibody in one or two absorptions. Considering those twenty sera with anti-cephalothin antibody to a titre of 1:16 or greater, eight showed no change in titre after two absorptions with penicillin sensitized erythrocytes and twelve showed at least a 2 tube decrease in titre.

The specificity of absorption was indicated by the fact that no diminution of anti-penicillin or anti-cephalothin antibody titre occurred when unsensitized erythrocytes were used for absorption. In addition, absorptions with penicillin and cephalothin sensitized erythrocytes failed to diminish the haemagglutination titre of a non-related haemagglutination system (anti-Rh_0).

Mercaptoethanol sensitivity

Seventy sera were treated with 2-mercaptoethanol (Table 7). Thirty-seven sera had positive haemagglutination titres for both penicillin and cephalothin sensitized erythrocytes, and thirty-three with one or the other drug.

After treatment with 2-mercaptoethanol, thirty of thirty-seven (81%) showed a complete loss of, two out of thirty-seven (5·5%) a partial reduction of, and five out of thirty-seven (13·5%) no change in haemagglutination with penicillin sensitized cells. Thirty-six of thirty-seven (97%) showed a complete loss and one out of thirty-seven (2·7%) a partial reduction of haemagglutination titre using cephalothin sensitized cells.

Sera from twenty-four patients demonstrating positive haemagglutination titres with penicillin sensitized red cells only, and from nine patients with positive titres toward cephalothin sensitized cells only, were similarly treated. Nineteen of twenty-four (79%)

and all of nine (100%) had a complete loss of haemagglutination titre and five out of twenty-four (21%) no change in titre.

Those sera with complete reduction of haemagglutination titre were thought to contain no IgG antibody, and those with no change or partial reduction of titre were classed as containing IgG antibody at least in part.

TABLE 7. The 2-mercaptoethanol sensitivity of anti-penicillin and anti-cephalothin antibodies

No. of patients	Erythrocytes resulting in haemagglutination before 2-ME treatment of serum	RBC used for haemagglutination after 2-ME treatment of serum	Complete loss of haemagglutination titre	No change or partial decrease in haemagglutination titre
37	Penicillin and cephalothin	Cephalothin sensitized	36/37 (97%)	1/37 (3%)
	Penicillin and cephalothin	Penicillin sensitized	30/37 (81%)	7/37 (19%)
24	Penicillin only	Penicillin sensitized	19/24 (79%)	5/24 (21%)
9	Cephalothin only	Cephalothin sensitized	9/9 (100%)	0

DISCUSSION

Cephalothin, a semi-synthetic derivative of 7 amino-cephalosporanic acid, is a broad spectrum antibiotic which is structurally related to the penicillins. Abraham & Newton (1961) and Abraham (1967) determined that the chemical structure of cephalothin contains fused β-lactam and dihydrothiazine rings, and noted that because of dissimilarities in the chemistry of the ring structures, cross-reactivity of penicillin and cephalothin probably would not occur *in vivo*.

Preliminary experiments by Schneierson, Perlman & Shore (1964) in rabbits, and skin tests in five persons with histories of hypersensitivity to penicillin by Stewart (1962) failed to reveal any evidence of cross-reactivity. Clinical reviews of fifty-two patients by Walters, Romansky & Johnson (1962), of eighty-nine patients by Weinstein, Kaplan & Chang (1964), of sixty-one patients by Griffith & Black (1964), and a report of 'clinical experience' in using the drug by Herrell, Balows & Becker (1963), did not reveal any allergic reactions in patients allergic to penicillin who received cephalothin.

However, Brandriss, Smith & Steinman (1965), Batchelor *et al.* (1966), and Shibata *et al.* (1966), demonstrated that cephalothin and cephaloridine conjugated to appropriate protein carrier molecules can induce the formation of antibodies in rabbits, detectable by haemagglutination, precipitation and passive cutaneous anaphylaxis, which cross-react with the major antigenic determinant of penicillin, i.e. the benzylpenicilloyl determinant.

The increased incidence of hypersensitivity reactions to cephalothin in persons with a history of hypersensitivity to penicillin (Merrill *et al.*, 1966; Thoburn, Johnson & Cluff,

1966) and the occurrence of allergic reactions upon initial injection of cephalothin in penicillin sensitive persons (Kabins, Eisenstein & Cohen, 1965; Turck *et al.*, 1965; Rothschild & Doty, 1966; Drug Letter II, 1966) suggests the possibility of cross-reactivity between cephalothin and penicillin.

However, such clinical data are not conclusive for several reasons. Smith, Johnson & Cluff (1966) have reported that in penicillin allergic individuals there is an increased incidence of hypersensitivity reactions to drugs unrelated to penicillin. In addition, we have recently reported immunological evidence for the development of specific acquired hypersensitivity to cephalothin but not to penicillin in a patient who had repeatedly received penicillin without adverse effect before receiving cephalothin (Abraham, Petz & Fudenberg, 1968).

Further, a severe allergic reaction within seconds of an intravenous injection of cephaloridine in a patient who had been treated previously with penicillin but not with cephaloridine was shown to be caused by environmental exposure to cephalosporin-C derivatives rather than to cross-allergenicity with penicillin (Kaplin & Weinstein, 1967). Environmental exposure, previously postulated as the cause of the high incidence of anti-penicillin antibodies in patients whether or not they have been treated with penicillin (Levine *et al.*, 1966b), may also be the cause of the anti-cephalothin antibodies in the group of normal persons reported herein who have never received cephalothin (Table 1).

This study provides experimental evidence that cephalothin is immunogenic as indicated by a comparison of the incidence of anti-cephalothin antibody (titre 1: 8 or greater) in controls and in patients receiving cephalothin. The incidence of anti-cephalothin antibody was 36% in 124 controls including patients who had been treated with penicillin or cephalothin for less than 3 days. As indicated in Table 4, patients receiving cephalothin from whom weekly serial samples were obtained (Group 2-Ceph) had an incidence of an anti-cephalothin antibody of 75% and an increase in titre of at least 2 tubes was noted in 25% of the patients.

Cross-allergenicity between cephalothin and penicillin is indicated by the fact that 81% of the patients in Group 2-Ceph had an anti-penicillin antibody (titre 1:8 or greater) compared with a 47% incidence in the 124 controls, and 25% of these patients had an increase in anti-penicillin antibody titre while receiving cephalothin. Further, of these patients who were treated with penicillin from whom weekly serial samples were obtained (Group 2-Pen) and who denied a history of cephalothin therapy, 69% had anti-cephalothin antibody (titre of 1:8 or greater) and 19% of these patients had an increase in anti-cephalothin antibody titre while receiving penicillin.

Absorption tests confirmed presence of cross-allergenicity. Absorption with cephalothin sensitized erythrocytes uniformly removed all haemagglutinating activity toward both cephalothin and penicillin sensitized erythrocytes. Greater specificity of the reactions was found using penicillin sensitized erythrocytes for absorption. Such erythrocytes removed all anti-penicillin antibody in one or two absorptions but had no effect on the anti-cephalothin antibody titre of eight of twenty sera with an original titre of 1:16 or greater. Even here, at least a 2-tube decrease in anti-cephalothin antibody titre was found after two absorptions in twelve of the twenty sera.

In addition, haemagglutination inhibition studies (Table 6) revealed cross-reactivity using both anti-penicillin and anti-cephalothin antibodies and cephalothin, cephaloridine, benzylpenicillin and penicilloyl polylysine as inhibitors.

The incidence of positive direct Coombs tests in persons receiving cephalothin has gained

recent attention. Gralnick *et al.* (1967) reported a positive Coombs test in eight of twenty (40%) patients and noted that all eight patients were both azotemic and hypoalbuminaemic. Molthan *et al.* (1967) reported a 75% incidence of positive direct Coombs tests; the blood urea nitrogen was measured in twenty patients and was elevated in thirteen. These workers presented evidence indicating that the positive Coombs tests were caused by non-specific absorption of serum proteins rather than as a result of an immune mechanism. Perkins *et al.* (1967) reported a 38% incidence of positive direct Coombs tests in patients receiving cephalothin; 44% of these patients were azotemic.

In the present study only eight of forty-nine patients receiving cephalothin were azotaemic and none of these had a positive direct Coombs test. Only one patient in the entire group developed a transiently positive (3+) test.

The incidence of a positive direct Coombs test in patients receiving intravenous penicillin has not previously been studied. Although penicillin is now the most common cause of drug-induced haemolytic anaemia for which there is serological evidence of a drug related antibody (Dausset & Contu, 1967), our findings of only three positive direct Coombs tests in 125 patients (2·4%) and no cases of haemolytic anaemia indicate that these are uncommon findings. However, the duration of penicillin administration before the final sample that we obtained for testing must be considered since a positive direct Coombs test will not occur until there has been sufficient time for the development of a high titre anti-penicillin antibody and for *in vivo* sensitization of the patient's erythrocytes by penicillin. In only nineteen of our 125 cases was the last sample obtained 10 or more days after initiation of therapy. It is probable that patients who have had frequent penicillin administrations in the recent past or who will be treated with intravenous penicillin for a matter of weeks will have a significantly higher incidence of immunohaematological abnormalities. Indeed, a large majority of the reported cases of penicillin induced haemolytic anaemia (Dausset & Contu, 1967) have occurred during therapy for bacterial endocarditis where prolonged therapy is usual.

The development of an antibody to a drug is only one prerequisite for the development of a drug-immune cytopenia. The drug and the anti-drug antibody must have physico-chemical characteristics which result in non-specific absorption of the antigen–antibody complex to one or another of the formed elements of the blood as is the case in most drug immune cytopenias (Shulman, 1963; Shulman *et al.*, 1964) or the drug itself must be firmly bound to the cell and serve as an antibody receptor site for the anti-drug antibody as in penicillin induced haemolysis (Petz & Fudenberg, 1966; Levine & Redmond, 1967). Our data and that of Levine *et al.* (1966c), indicate that in patients who have penicillin induced Coombs positivity, penicillin can be demonstrated on the patient's erythrocytes using a rabbit anti-penicillin antiserum and antibody is demonstrable using an anti-immunoglobulin serum. Indeed, this 'double Coombs test' may be used as an aid in diagnosis.

Similar information is needed concerning cell binding of cephalothin and anti-cephalothin antibody. Lee & Anderson (1962) have reported that in dogs cephalothin seems to be loosely bound to erythrocytes since the drug can be removed from the erythrocytes in three or less washes with normal saline. We have tried to detect *in vivo* binding of cephalothin to human erythrocytes by use of a rabbit anti-cephalothin antiserum. In tests of erythrocytes from twenty-seven patients receiving cephalothin, we obtained only two positive results; both patients had an elevated blood urea nitrogen.

These preliminary results need confirmation and extension since in the absence of sufficient absorption by erythrocytes of cephalothin alone or of cephalothin–anti-cephalothin–antibody complex, drug-immune haemolysis will not occur even when a high titre of anti-cephalothin antibody is present. This may explain why there are as yet no reports of cephalothin-induced haemolytic anaemia. Further work on this problem is in progress.

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