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Direct Coombs' Test Reactivity after Cephalothin or Cephaloridine in Man and Monkey (33330)

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The development of positive direct Coombs' reactions (DCR) in humans during administration of drugs has attracted increasing attention in recent years. This association can be of practical importance since in some instances the Coombs' reactivity may also be associated with hemolytic anemia; notable examples have included methyldopa and penicillin (1–3). Preliminary studies indicate that 40–70% of patients given cephalothin may develop positive DCR (4,5). This unusually high incidence emphasizes the need to define possible mechanisms of red blood cell alteration or sensitization, and to establish any association with red cell damage that might influence their survival. Although structurally similar to cephalothin (6), cephaloridine has been reported to have caused a positive DCR in only one patient (7).

The present study was conducted to determine (1) the incidence of positive DCR, (2) relationships to serum antibiotic concentrations, and (3) possible associated red blood cell abnormalities in patients and rhesus monkeys treated with cephalothin or cephaloridine.

Materials and Methods. During the period January through June, 1967, 143 patients at University Hospital were observed during cephalothin therapy. Serial DCR were performed by washing 2% suspensions of red blood cells three times in normal saline in 10 × 75-mm tubes, decanting the supernatant fluid; 2 drops of Coombs' anti-human serum (Pfizer) were added and the contents centrifuged for 15 sec in a Clay-Adams serofuge. DCR were interpreted as 4+ to 1+ depending upon the degree of macroscopic agglutination; microscopic agglutination was recorded as ± and considered insignificant. Serial hemoglobin (cyanmet-hemoglobin) and hematocrit (micromethod) determinations were obtained twice weekly during cephalothin treatment (8).

The comparative effect *in vitro* on DCR of human and monkey erythrocytes exposed to graded concentrations of cephalothin or

cephaloridine was also studied; 0.5 ml of blood was added to 0.5-ml dilutions of the antibiotics in normal saline and incubated for 4 hr at 37°. The erythrocytes were then washed three times and resuspended in saline, and DCR determined as described above.

The effect of cephaloridine on the DCR was evaluated in normal humans; 2 groups of 5 volunteers were given 0.5 and 1.0 g, respectively, intramuscularly every 8 hr (25–50 mg/kg/day) for 15 days and serial DCR obtained. Additional screening tests, including complete blood counts, urinalysis, serum glutamic oxalacetic transaminase, lactic dehydrogenase, blood urea nitrogen (BUN), and creatinine, were performed to exclude toxicity.

A total of 34 rhesus monkeys were given cephalothin or cephaloridine 100–150 mg/kg/day, intramuscularly or intravenously in 12-hour divided doses until a positive DCR occurred or for a maximum treatment period of 24 days if the DCR remained negative. Four control monkeys were given penicillin G 100–150 mg/kg/day, intramuscularly. In a separate experiment, 2 groups of 2 monkeys each were given cephalothin or cephaloridine 100 mg/kg/day, intramuscularly, in 12-hr divided doses for 28 days during which time erythrocyte determinations were obtained at 2–3-day intervals. Two control monkeys were untreated. Coombs' anti-human serum (Pfizer) was employed in these tests in view of the homology between human and macacus plasma proteins, particularly the gamma globulins (9).

In the monkeys, serial hematocrits were performed by a micromethod and hemoglobin determined as cyanmethemoglobin (8). Red blood cells were enumerated in a Coulter model F counter, blood films stained and examined for Heinz bodies, and reticulocytes counted by standard methods (8). Osmotic fragility of red blood cells was studied using the Fragiligraph developed by Danon; values were expressed as the percentage of NaCl at which 50% lysis of cells occurred (10). Reduced glutathione was determined by the method of Beutler (11). Erythrocyte adenosine-triphosphate (ATP) was determined as described by Beutler (12), and autohemolysis was studied at 37° (13). Erythrocyte acetylcholinesterase and glycolytic enzymes were determined by established procedures (14–17).

TABLE I. Duration of Cephalothin Therapy and Onset of Positive Direct Coombs' Reactions in 42 Patients.

Day first positive DCR	Number of patients
1	2
3	6
4–6	6
7–10	8
11–14	7
15–21	10
22–28	1
33	1
38	1

Serum antibiotic concentrations were determined concomitantly on blood samples tested for Coombs' reactivity and were obtained by the cup-plate method employing *Sarcina lutea* as the test organism (6).

Results. Clinical and volunteer studies. The DCR became positive in 54 (38%) of 143 patients treated with cephalothin. The time of onset of positive DCR after the initial antibiotic injection could be accurately determined in 42 of the 54 patients. As shown in Table I, this ranged from 1–38 days (mean 15 days). The relationship of cephalothin dosage to Coombs' reactivity is shown in Table II. The average daily and total doses did not differ significantly in the Coombs' positive and negative patient-groups. In addition, there was a wide range in the amount of cephalothin received by the time of the first positive DCR. In 12 patients the DCR became negative in 1–14 days (mean 6.4 days) after cephalothin therapy was discontinued. In 1 patient the DCR became negative during continued therapy. In the remaining 41 patients with a positive DCR, the time of reversion to a negative reaction could not be determined accurately because of discharge from the hospital, death, or inability to obtain appropriate blood samples. There was no positive correlation between serum concentrations of cephalo-

TABLE II. Relation of Cephalothin Dosage in 143 Patients to Direct Coombs' Reactivity.

	Positive DCR 54 patients (range and mean)	Negative DCR 89 patients (range and mean)
Grams per day	2–10 (4.4)	2–12 (4.2)
Total grams received	3–172 (52)	4–194 (46)
Grams received at time of first positive DCR	3–156 (39)	

thin achieved and the development of a positive DCR. As shown in Fig. 1, various high and low serum cephalothin levels were associated randomly with either positive or negative DCR. An elevated BUN (>20 mg/100 ml) was present in 24 of the 54 patients who developed positive DCR (mean BUN 34 mg/100 ml; range 21–80 mg/100 ml) and in 39 of the 89 patients with negative DCR (mean BUN 25 mg/100 ml; range 5–57 mg/100 ml). Hypoalbuminemia (<3 g/100 ml) was observed in 7 and 3 patients with positive and negative DCR, respectively. The occurrence of anemia attributable to cephalothin therapy was not observed.

In the 2 groups of 5 volunteers given cephaloridine 1.5 g/day or 3.0 g/day for 15 days, the ranges of serum concentrations were 2.6–56.5 μg/ml and 7.7–54.3 μg/ml, respectively. One of the 5 receiving 3.0 g/day developed a positive DCR on day 14 that persisted for 1 week. This volunteer had a slightly elevated BUN (25 mg/100 ml) and the range of cephaloridine concentrations in his serum was 9.8–40.0 μg/ml. No other adverse reactions were observed in the volunteers.

Rhesus monkey studies. Six of 14 monkeys given cephalothin 100–150 mg/kg/day, intramuscularly, developed positive DCR after 3–17 days (Table III). Similar intramuscular doses of cephaloridine resulted in positive DCR in 13 of 14 animals after 5–21 days. Cephalothin 100 mg/kg/day, intravenously, produced positive DCR in 2 of 3 monkeys in 7 and 16 days. The same intravenous dose of cephaloridine resulted in all 3 becoming positive in 7 days. No anemia was observed in the monkeys with positive DCR and blood films and reticulocyte counts remained normal. There were no significant changes of osmotic fragility or autohemolysis. Red cell ATP and glycolytic enzymes remained normal and no evidence of overload of the hexosemonophosphate shunt was observed.

TABLE III. Direct Coombs' Reactivity in Monkeys after Cephalothin or Cephaloridine.

Dosage (mg/kg/day)	Route	Positive DCR	Days to positive DCR
Cephalothin			
100	i.m.	4/9	3–17 (mean 9.2)
150	i.m.	2/5	3, 12 (mean 7.5)
100	i.v.	2/3	7, 17 (mean 12)
Cephaloridine			
100	i.m.	9/9	5–21 (mean 10.7)
150	i.m.	4/5	4–11 (mean 8.0)
100	i.v.	3/3	7, 7, 7 (mean 7.0)
Penicillin G			
100	i.m.	0/2	
150	i.m.	1/2	9

The comparative ability of cephaloridine and cephalothin to induce positive DCR with monkey and human erythrocytes was further assessed by *in vitro* studies shown in Table IV. A positive DCR was observed with monkey red cells after exposure to cephaloridine concentrations as low as 250 μg/ml, but human cells required 500 to 1000 μg/ml.

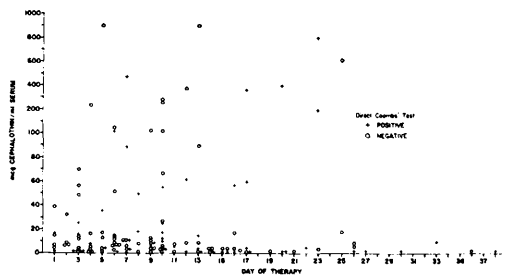

FIG. 1. Coombs' reactivity and serum cephalothin concentrations in relationship of cephalothin serum concentrations, day of cephalothin treatment, and direct Coombs' reactivity in patients.

TABLE IV. Direct Coombs' Reactivity after *in Vitro* Exposure of Erythrocytes to Cephalothin or Cephaloridine.

	Dose (mg/ml)	Direct Coombs' reactions[a]	
		Human RBC	Monkey RBC
Cephaloridine	2.0	+	+++
	1.0	+	++
	.5	±	++
	.35	—	++
	.25	—	+
	.125	—	±
Cephalothin	2.0	++	++
	1.0	++	++
	.5	+	+
	.35	+	+
	.25	±	+
	.125	—	±

[a] ± = microscopic agglutination only; interpreted as negative; +, ++, +++ = degrees of macroscopic agglutination; positive tests.

Cephalothin produced a positive DCR at 250 to 350 μg/ml with erythrocytes from both species.

Discussion. The data presented confirm that positive DCR may occur in approximately 40% of cephalothin-treated patients; the same incidence was also observed in normal monkeys given cephalothin. This reaction in patients does not appear related to dose or serum concentration, but may tend to occur more frequently in those with elevated BUN (4). Specifiically, anemia attributable to cephalothin was not observed in patients or monkeys. The preliminary study with low doses of cephaloridine in monkeys suggests that a higher incidence of positivity may occur, but, *in vitro* experiments (Table IV) indicate that monkey red blood cells may be more easily sensitized than those from a human source. As with cephalothin, anemia was not observed in volunteers or monkeys given cephaloridine.

In a separate study (unpublished data) 3 groups of 3 monkeys each received 100 mg/kg/day of cephalothin or cephaloridine, intramuscularly, and cephalexin (19), orally; 1 of 3 receiving cephalothin and 1 of 3 receiving cephaloridine developed positive DCR on day 8 and day 11, respectively. Similarly, 1 of 3 receiving cephalexin, orally, developed a positive DCR on day 11. In contrast, in a study of experimental staphylococcal infections in monkeys (unpublished data) comparing the efficacy of 100 mg/kg/day of cephalothin and cephaloridine, intramuscularly, and cephalexin, orally, only 1 of 36 monkeys developed positive DCR; this animal received cephalothin for 9 days after intravenous bacterial challenge and showed positive DCR from day 8 through day 14.

The ultimate significance of Coombs' reactivity in man caused by either cephalothin or cephaloridine is unclear at this time as is the higher incidence of DCR in normal monkeys as compared to that in animals with staphylococcal sepsis. The measures of monkey erythrocyte membrane and biochemical integrity in the present studies showed no detectable evidence of a damaging effect, although no direct determinations were made of red cell survival. Further assessment by ultramicroscopic methods of possible red cell membrane effects are in progress. Currently, no data have appeared to indicate that positive DCR in man associated with cephalothin treatment of infection has resulted in deleterious effects on red blood cells. However, single reports of Coombs' positive hemolytic anemia probably due to cephaloridine (7), and cross-reactivity of cephalothin-coated erythrocytes in a penicillin-allergic patient (18) should encourage further observations regarding the possibility of hemolysis in patients with cephalosporin-induced Coombs' reactivity.

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Immunodiffusion Reactions between Human Sera and *Mycoplasma pneumoniae** (33331)

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Mycoplasma pneumoniae is an important respiratory tract pathogen of man. Although this microorganism can be present in individuals who are asymptomatic, its effects may range from mild respiratory disease to primary atypical pneumonia characterized by the development of cold hemagglutinins (1, 2).

Current procedures for isolation and identification of *M. pneumoniae* require the use of complex culture media and prolonged incubation periods (3, 4). Consequently, many investigators have relied upon serologic procedures to obtain evidence of *M. pneumoniae* infection. Tests used to detect *M. pneumoniae* antibody in human sera include complement-fixation, immunofluroescence, hemagglutination, and growth inhibition procedures (5, 6).

The tetrazolium reduction inhibition test (TRI) is a sensitive means of measuring growth inhibiting antibody against *M. pneumoniae*. If specific antibody is present, the organism does not grow and the tetrazolium dye indicator is not reduced. Growth inhibiting antibody has been correlated with resistance to illness caused by *M. pneumoniae* (7). Volunteers infected with *M. pneumoniae* were protected against illness when detectable levels of growth inhibiting antibody were present prior to challenge. In addition, growth inhibiting antibody at a level of 1:16 or greater prevented infection. Antibody responses to *M. pneumoniae* vaccines are evaluated frequently by the TRI test (8, 9). This test requires a 5–6 day incubation and can be invalidated by bacterial contamination or nonspecific inhibitors in the serum. Double diffusion gel precipitation (ID) techniques are not subject to these limitations.

Previously, Taylor-Robinson *et al.* (10) demonstrated ID reactions with several animal mycoplasmas and hyperimmune animal sera. However, they experienced difficulties in obtaining a satisfactory *M. pneumoniae* ID antigen. Even with improved methods for

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