zygous Hb C disease show abnormal physical properties in vitro—their viscosity is higher than that of normal cells and they appear to be more rigid. These differences are exaggerated when the osmolality of the suspending solution is increased (Charache *et al.*, 1967). Given these in-vitro observations, it may be postulated that alterations in the physical properties of the red cells of this patient produced by the hyperosmolar state may have resulted both in ischaemic mononeuritis multiplex due to occlusion of vasa nervorum and acute renal failure due to intravascular damage or sludging within the renal glomeruli.

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Massive Haemolysis Caused by Rifampicin

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Rifampicin has been shown to be a highly effective anti-tuberculosis drug and is now being used in initial therapy as well as in retreatment regimens (Favez *et al.*, 1972). The serious side effects with daily therapy have been minimal and have mainly consisted of hepatotoxic reactions (Lees *et al.*, 1970). A higher incidence of adverse reactions has been reported with high dose intermittent rifampicin (Poole *et al.*, 1971, Aquinas *et al.*, 1972). These reactions—thrombocytopenia and febrile episodes—were associated with rifampicin-dependent antibodies in the serum (Poole *et al.*, 1972). Although these antibodies fix complement to red blood cells, haemolytic reactions have been reported in two instances only (Hasse *et al.*, 1971, Sors *et al.*, 1972). This paper reports a case of near fatal massive haemolysis caused by rifampicin.

Case Report

A 54-year-old woman was admitted to Colorado General Hospital on 8 May 1972 after an overdose of glutethimide and alcohol for which she had been treated at another hospital for five days with supportive care and assisted ventilation. At the time of admission she was conscious and had a tracheostomy tube in position.

Pulmonary tuberculosis was first diagnosed in 1930 and recurred in 1942; both episodes were treated with bed rest. In 1962 sputum cultures for *Mycobacterium tuberculosis* were positive and she received a six-month course of isoniazid and streptomycin. Her sputum was again positive by culture in March 1970, and she was treated with isoniazid, pyrazinamide, and ethambutol. She then developed migratory arthritis secondary to isoniazid and this drug was discontinued. While still receiving pyrazinamide and ethambutol she had a positive culture for *Myco. tuberculosis* in May 1971. Based on in-vitro susceptibility studies and previous drug therapy, she was given rifampicin 600 mg/day, ethionamide 500 mg/day, and kanamycin 1 g/day. After three months the kanamycin was discontinued and she was discharged from the hospital in September 1971. By her own admission, she took

rifampicin irregularly and discontinued the ethionamide in December 1971 because of gastrointestinal intolerance. After her drug overdose, rifampicin alone was resumed on 5 May 1972 but was again discontinued on 8 May, when she was admitted to this hospital.

On 14 May, rifampicin and ethambutol were resumed. Two hours after receiving the drugs the patient was found to be hypotensive and unresponsive. She was transferred to the intensive care unit, where a diagnosis of severe haemolysis was established on the basis of a serum haemoglobin of 510 mg/100 ml, absent serum haptoglobins, a fall in the packed cell volume of 20 volumes %, raised indirect serum bilirubin, and haemoglobinuria. The platelet count was 155,000/mm³ compared to 350,000/mm³ six days earlier. Other liver function tests showed no abnormality and the serum total haemolytic complement levels were consistently less than 20 CH₅₀ units/ml (50% haemolytic unit of complement) for three days (normal 33-61 CH₅₀ units/ml). Blood cultures were sterile on several occasions. The patient became anuric after this haemolytic episode and required haemodialysis on four occasions, with subsequent recovery of her renal function. During the haemolytic episode her direct Coombs test was strongly positive with a negative indirect test.

After her recovery, as the aetiology of her haemolytic episode was not established and because of the desirability for further antituberculosis therapy, the patient was challenged with increasing doses of rifampicin while following her closely for evidence of haemolysis. She was given 10, 20, 50, 100, 200, and 300 mg of rifampicin on successive days with daily monitoring of packed cell volume, reticulocyte count, platelets, serum bilirubin, plasma haemoglobin, and urinary haemoglobin levels. Two hours after a dose of 300 mg her temperature rose from 36.5 to 38.2°C orally and she developed a shaking chill. Her serum bilirubin and plasma haemoglobin levels were raised from baseline values. Her packed cell volume fell by 6 volumes % and her serum haptoglobin fell by 79 mg/100 ml. The platelet count fell from 260,000 to 208,000/mm³; urinary haemoglobin was also detected (table I). Her blood pressure was stable. At the time of the trial doses of rifampicin she was not receiving any other medication.

TABLE I—Results after Challenge with 300 mg Rifampicin

	P.C.V.	Serum Bili-rubin Total/Direct (mg/100 ml)	Serum Hapto-globins (mg/100 ml)	Urinary Haemo-globin	Platelet Count/mm ³	Plasma Haemo-globin (mg/100 ml)	Temp-erature (°C)
Baseline values	29%	0.7/0.2	128	—	260,000	<1 mg	36.5
Two hours after 300 mg rifampicin	23%	3.2/0.5	49	+	208,000	21 mg	38.2

At the time of writing she was not on antituberculosis drugs as her sputum had remained consistently negative for mycobacteria on culture.

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SPECIAL STUDIES

Direct and indirect antiglobulin studies were carried out on the patient's blood using the method described by Harris (1956). The direct test gave a strong agglutination with anti-complement serum indicating that the patient's red cells were coated with C₃ complement. Negative reactions were obtained with anti-IgG, anti-IgA, and anti-IgM serum.

The indirect tests were performed after the addition of rifampicin solution (7 mg pure rifampicin in 10 ml saline) to the patient's serum. Equal amounts of rifampicin solution and serum were incubated at 37°C for half an hour. Rh-negative group O red blood cells were then incubated with the mixture for one hour and read with a commercial broad spectrum Coombs reagent, anti-IgG, anti-IgM, anti-IgA, and anti-complement sera. Serial dilutions of the various antisera up to 1:512 were used. Control tests were performed with saline substituted for rifampicin and antihuman serum albumin. A positive reaction was obtained only with the anti-complement serum (see table II). All control tests yielded negative results.

TABLE II—Results of Direct and Indirect Antiglobulin Tests

Test Sample	Antiglobulin Reaction with				Com- mercial Coombs's Reagent
	Anti- IgG	Anti- IgM	Anti- IgA	Anti- IgC ₃	
Red cells (direct Coombs test) ..	—	—	—	+++	—
Serum and normal red cells and saline ..	—	—	—	—	—
Serum + normal red cells + rifampicin solution* ..	—	—	—	+++	—

*Maximum reaction at 1/8 dilution of antiserum, positive reactions through 1/128 dilution, negative reactions with 1/256 and 1/512 dilutions.

The serum for the indirect tests was separated from the red cells within a few hours of taking the sample and frozen to -70°C before testing. Rh-negative group O red cells were used as the patient was Rh-negative group O. Several samples of the patient's serum obtained on different days, including the day of the haemolytic reaction, were tested and they all gave similar results.

Comment

The presence of rifampicin-dependent antibodies which bind complement to the surface of the patient's own red cells and platelets has been found in patients receiving high-dose (1,200 mg) intermittent rifampicin therapy (Poole *et al.*, 1971). Though thrombocytopenia and febrile reactions were associated with the presence of these antibodies, no clinical haemolysis was seen.

In our patient, a well-documented massive haemolytic episode occurred after she had been receiving rifampicin for nearly a year. For the latter eight months, however, she had been taking the drug irregularly and hence may have been receiving something akin to an intermittent regimen. In addition, as her weight was 40 kg, the standard 600-mg dose of rifampicin was relatively high for this particular patient.

The mechanism of this drug-induced haemolysis may be similar to that of quinine-induced haemolytic anaemia (Croft *et al.*, 1968). The drug-related antibody; in the presence of drug in the serum, fixes and activates complement on the surface of the red cell. This leads to destruction of the red cell. In our patient appreciable amounts of complement were found using the direct antiglobulin test with specific antisera. The patient's serum fixed complement to the surface of normal red cells in the presence of drug but not in its absence. No immunoglobulins were found in the indirect antiglobulin test using serial dilutions of the antisera up to 1/512. However, Gilliland *et al.*, (1970) found, by special techniques, appreciable amounts of 7S IgG molecules on the surface of red cells showing positive direct antiglobulin tests with anti-complement serum and negative reactions with antisera to IgG, IgA, or IgM. They used a complement fixing antibody consumption test. It is also possible that the antibody involved may be a small amount of a high efficiency IgM as has been postulated by Croft *et al.*, (1968). Our inability to identify the drug-related antibody is probably due to the relative insensitivity of the techniques used.

With the widespread and increasing use of rifampicin the possibility of a potentially fatal adverse effect should be recognized.

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