and chloramphenicol acetylase (for review see Davies, and Rownd 1972). Such enzymes may possibly have evolved to protect bacteria against naturally occurring antibiotics. But the R factors first identified, as well as many recognized later all over the world, give resistance to sulphonamides as well as to antibiotics.

The mechanism of R-factor-mediated sulphonamide resistance is not known. Can it have evolved since the first use of sulphonamides in the 1930s? It seems unlikely. More probably a biochemical mechanism protective against sulphonamides already existed, evolved in response to unknown pressures, and was selected and encouraged to spread by the medical use of sulphonamides. Previously known R factors carrying genes for sulphonamide resistance do not, in our experience, confer resistance to trimethoprim. Lebek and Wiedmer (1971) and Lebek (personal communication) found, however, that some such R factors conferred a low level of trimethoprim resistance. The R factors we describe here give resistance to both drugs (as well as, in one case, to unrelated antibiotics). We do not know whether separate genes control resistance to the two synthetic drugs or whether a single biochemical mechanism gives resistance to both.

Resistance to trimethoprim was found in 1% of strains of *E. coli* isolated in 1966 from urinary infections in general practice two years before the drug became generally available for medical use. The prevalence of trimethoprim resistance in clinical isolates of *E. coli* remains at about this level in both hospital and domiciliary practice (Grüneberg, 1971). The prevalence of trimethoprim resistance in *Klebsiella* spp. in hospital is higher (33%) than in *E. coli*. It is not known how much of the trimethoprim resistance at present found in enteric bacteria is R-factor determined.

The widespread use of trimethoprim may encourage the spread of such R factors, but up until now no such spread has become apparent in *E. coli*. R factors giving resistance, especially

to streptomycin, the tetracyclines, and sulphonamides, are common in the normal bacteria of the human intestine (Datta, 1969; Datta *et al.*, 1971). All these drugs have been in use for over 20 years. Infections caused by bacteria resistant to them occur, and the proportion of R+ urinary infections outside hospital has increased, though not appreciably so, during the past decade (Shaw *et al.*, 1972; Gillespie *et al.*, 1971). What will happen in respect of trimethoprim-resistant R factors can at present only be a matter for speculation. We hope that clinical bacteriologists will monitor and report any changes which may occur in the usefulness of this valuable drug.

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MEDICAL MEMORANDA

Haemolytic Anaemia associated with Antibodies to Tolbutamide and Phenacetin

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Several drugs are known to give rise to a positive direct Coombs test with or without haemolytic anaemia (Pirofsky, 1969; Robinson and Faodi, 1969; Stefanini and Johnson, 1970; Henry *et al.*, 1971). There are at least three types of drug-dependent positive direct Coombs tests: (1) the stibophen type, in which the drug combines with antibody and the complex is adsorbed non-specifically on to the red cells and fixes complement; (2) the penicillin type, where the drug is readily

attached to red cells, which are then either directly agglutinated by anti-penicillin antibody or, if the antibody is weak, shown by a positive indirect Coombs test; and (3) the Aldomet (methyldopa) type, in which methyldopa either alters the red cell membrane or affects the antibody-forming tissues so that an autoantibody is produced which has a specificity within the rhesus blood group system. The drug takes no part in the serological reaction. Robinson and Faodi (1969) distinguished a fourth or cephalothin type; cephalothin, however, probably operates by mechanisms 1 and 2.

We here report the case of a patient with an immune haemolytic anaemia associated with tolbutamide, a sulphonylurea compound, and phenacetin.

Case Report

The patient, an Italian woman aged 58, presented with a history of lassitude, dyspnoea, headaches, and weakness of the legs for four weeks. Routine blood tests on 9 July 1971 showed haemoglobin 7.4 g/100 ml, P.C.V. 22%, M.C.H.C. 33%, and reticulocytes 30.5%. A blood film showed pronounced anisocytosis, poikilocytosis, and polychromasia, with normal white cell morphology. Haemolytic anaemia was suspected. She was admitted to hospital on 11 July and on examination appeared anaemic.

There was no evidence of bruising, lymphadenopathy, splenomegaly, or hepatomegaly, and the finger-nails were normal. Her pulse rate was 90/min and regular, blood pressure 130/90, and there were no other appreciable clinical features. A full blood investigation on 14 July showed: haemoglobin 7.8 g/100 ml;

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W.B.C. 7,700/mm³ (polymorphs 70%); P.C.V. 22%; M.C.H.C. 35%; platelets 284,000/mm³; reticulocyte count 31.5%; serum bilirubin 1.1 mg/100 ml; direct bilirubin less than 0.5 mg/100 ml; serum haptoglobin negative; haemoglobin electrophoresis, no abnormal haemoglobins; haemoglobin A₂ 1.8%; and Sickledex test for haemoglobin S negative. Osmotic fragility was normal; haemolysis started at 0.6% NaCl and was complete at 0.4% NaCl (control started at 0.5% NaCl and was complete at 0.35% NaCl). The blood urea was 41 mg/100 ml, serum iron 124 µg/100 ml, and total iron binding capacity 408 µg/100 ml. The direct Coombs test was strongly positive. Examination of the faeces showed no occult blood and the urine showed a trace of glucose and no other abnormalities. X-ray examination showed slight cardiac enlargement with normal lung fields. Her daughter was screened for evidence of haemoglobinopathy, with negative results.

Tolbutamide therapy had been stopped on 11 July and metformin 500 mg three times a day substituted. On 24 July the haemoglobin was 7.0 g/100 ml and the reticulocyte count 50%. In view of these findings steroid therapy (prednisone 60 mg daily) was introduced. By 12 August the haemoglobin had risen to 12.2 g/100 ml and the reticulocyte count had fallen to 26%. Prednisone was reduced to 40 mg daily and the patient continued to make satisfactory progress. She returned to Italy at the end of August, at which time the haemoglobin was 14 g/100 ml and the reticulocytes 5%.

SEROLOGICAL FINDINGS

A blood specimen was referred to the Transfusion Service on 15 July. Investigation showed that though the direct Coombs test was strongly positive no blood group antibody was demonstrable in the serum. This suggested the possibility of a drug-dependent antibody. On inquiry it was ascertained that the patient had been on tolbutamide (0.5 g three times a day) for 14 months. The investigation was therefore directed towards tolbutamide.

In preliminary tests 1 volume of 5% Rastinon intravenous diagnostic diabetes test solution was added to 2 volumes of packed normal red cells and incubated at 37°C for 30 minutes, washed four times, and resuspended in physiological saline solution to make a 10% suspension. The patient's serum was found to agglutinate tolbutamide-treated cells but not untreated cells from the same donor. Normal serum did not agglutinate tolbutamide-treated cells. Previous exposure of the patient's serum to tolbutamide for 30 minutes inhibited the reaction. The antibody was best shown by the tile technique. Agglutination was slow to develop, becoming appreciable in about 30 minutes and very strong in about one hour. It was of course necessary to place the tile under a moist cover to prevent drying of the cell-serum mixture. Subsequent tests with a 5% solution of Rastinon prepared without the other ingredients of Rastinon solution for the diagnosis of diabetes gave the same results. Agglutination of tolbutamide-treated cells was shown to be due to IgG antibodies. Agglutination of tolbutamide-treated cells did not require complement; the IgG antibodies were demonstrable by indirect Coombs tests using anti-IgG sera. Further tests showed that the antibody cross-reacted with red cells treated with chlorpropamide and glibenclamide.

It was then learnt that the patient was a regular user of phenacetin (and occasionally aspirin) because of frequent headaches. A further specimen investigated on 26 July showed a much weaker positive direct Coombs test and no free antibody was demonstrable in the serum. The serum was therefore concentrated and shown to contain antibodies active against both tolbutamide-treated and phenacetin-treated cells. Absorption with tolbutamide-treated cells removed antibody for phenacetin-treated cells and vice versa. No antibody for cells treated with acetylsalicylic acid was demonstrable.

Tolbutamide and phenacetin appear to bind much more loosely than penicillin with the red cell surface and all serological manipulations therefore required great delicacy.

Regrettably the patient returned to Italy before the investigation could be extended to various other drugs structurally related to tolbutamide and phenacetin.

antibody, apparently of the stibophen type, was found in the patient's serum. In the presence of chlorpropamide and complement it readily haemolyzed the red cells of patients with paroxysmal nocturnal haemoglobinuria. The antibody was shown to cross-react with tolbutamide and other sulphonylurea compounds. It is clear from our observations and those of Logue *et al.* that sulphonylurea compounds must be added to the steadily increasing number of therapeutic substances which can induce haemolytic anaemia.

An interesting if awkward complication was the demonstration of cross-reacting antibody to phenacetin. We are unable to say if the antibody was primarily an antibody to phenacetin which cross-reacted with the sulphonylureas or primarily an antibody to tolbutamide which cross-reacted with other sulphonylureas and with phenacetin.

The chemical structures of phenacetin and the various sulphonylureas studied are shown in the Fig. All these materials are substituted benzene compounds. Besides phenacetin and the sulphonylureas other benzene derivatives—for example, para-aminosalicylic acid (MacGibbon *et al.*, 1960; Dausset and Bergerot-Blondel, 1961) and dipyrone (Lay, 1966)—have been implicated in immune haemolytic anaemias. There is appreciable evidence therefore that some substituted benzene compounds are potentially immunogenic.

Chemical structure of phenacetin and some sulphonylureas.

The cross-reaction of the tolbutamide and phenacetin antibodies may be based on the chemical similarity of the two drugs. On the other hand, the two antibodies may have developed quite independently and their apparent interaction may not be dependent on the chemical similarity of the inciting antigens. The only other example of a phenacetin antibody which apparently cross-reacted with another antibody is that described by Hart and Mesara (1969), in which phenacetin antibody cross-reacted with an autoimmune erythrocyte antibody. The cross-reaction was probably based not on a chemical similarity between phenacetin and erythrocyte antigen but the formation of a phenacetin-autoantibody complex on the erythrocyte surface. It is possible that a similar mechanism may have been responsible for the apparent cross-reaction of the tolbutamide and phenacetin antibodies in the present case.

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Comment

Logue *et al.* (1970) reported an immune haemolytic anaemia due to the antidiabetic compound chlorpropamide. An IgG