Inhibition of red cell agglutination in the ABO system by promethazine

SIR,—We observed that the addition of promethazine hydrochloride to normal human serum delayed the rate of agglutination of red cells containing the corresponding antigens, particularly in the ABO blood group system (Barrie & Tait, 1967). Since promethazine crosses the placental barrier, it is possible that if administered during pregnancy it might prevent cell destruction in the foetus in the case of blood group incompatibility between mother and infant. Biermé & Biermé (1967), in fact, have reported that promethazine had been used with apparent success in conjunction with intraperitoneal transfusion in the treatment of hydrops foetalis due to Rh incompatibility. I have therefore examined further the effect of promethazine on red cell agglutination.

Because high concentrations of promethazine cause haemolysis of erythrocytes (Seeman & Weinstein, 1966), preliminary tests were made to determine the concentration producing haemolysis. A sample of 0·2 ml of a suspension of Group A red cells in 0·9% saline was added to 3·8 ml of distilled water and the haemolysed mixture was adjusted to give a colorimeter (EEL) reading of 3·5, corresponding to a haemoglobin value of 1·02 g/100 ml suspension. Equal volumes (0·75 ml) of drug solution (10^{-1} to 10^{-4} M in 0·9% saline) and of Group A cell suspensions were mixed in haemagglutination tubes (9 cm × 0·7 cm) and allowed to stand at 20° for 30 min. After centrifugation, the haemoglobin content of 1 ml of supernatant was measured spectrophotometrically and units of haemoglobin read from a standard curve obtained from cells of known haemoglobin content (Red Cross Blood Transfusion Service, Sydney). The minimum final concentration of promethazine producing haemolysis was between 0·125 and 0·25 × 10^{-2} M. Using serum in place of saline gave a slightly higher end point (0·25 — 0·375 × 10^{-2} M) possibly due to a lower fragility of red cells in serum. The haemolytic effect of promethazine was independent of concentration of red cells in suspensions ranging from 2 to 10%.

The effects of promethazine on agglutination were investigated in a system consisting of Group A or Group B red cell suspensions standardized to a haemoglobin value of 1·02 g/100 ml and mixed Group O serum standardized to contain approximately constant anti-A and anti-B titres. Solutions of promethazine in 0·9% saline containing 0·75 × 10^{-2}, 3 × 10^{-3}, 3 × 10^{-4}, 3 × 10^{-5} and 3 × 10^{-6} M of drug were prepared. Drug solution (0·25 ml) was added to 0·50 ml of serum and mixed thoroughly with 0·75 ml of cell suspension in a haemagglutination tube. Control tests were made with undiluted serum and with 0·9% saline. The tubes were stood for 30 min at 15–20° during which time the agglutinated cells settled. The proportion of agglutinated cells was determined by measuring the haemoglobin content of the supernatant which contained the unagglutinated cells. An aliquot of 1 ml of the upper portion of supernatant was removed (without centrifugation) and was added to 4 ml of distilled water to haemolyse the red cells present and the haemoglobin value was estimated spectrophotometrically. The addition of promethazine to serum invariably produced a fine white precipitate. Samples were therefore filtered immediately before taking spectrophotometric readings.

The percentage of agglutinated cells are plotted against promethazine concentration in Fig. 1. The highest concentration used was 1·25 × 10^{-3} M, which was approximately the concentration causing the first appearance of haemolysis. This suggests that without the interference of haemolysis even greater inhibition of agglutination may have occurred with higher concentrations. The threshold concentration of promethazine for inhibition of agglutination was 5 × 10^{-5} M. Similar results were obtained when solutions of promethazine

were prepared from ampoules (25 mg/ml). Since saline solutions of promethazine hydrochloride (pure substance) rapidly deteriorated on standing in the light, it was more convenient to use the ampouled drug.

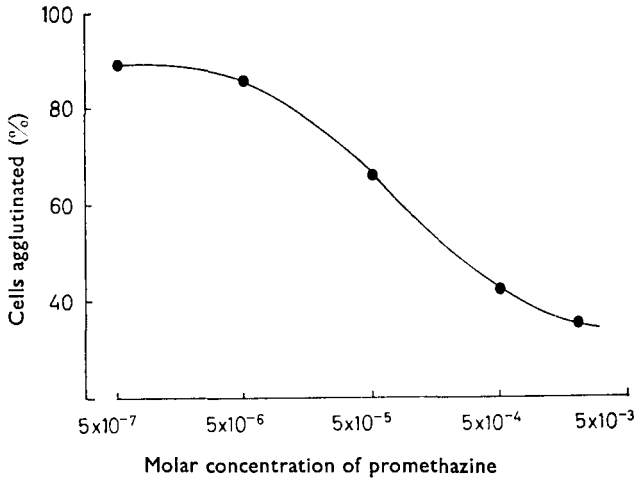

FIG. 1. The range of concentration of promethazine producing inhibition of agglutination of a Group A cell suspension by Group O serum. Molar concentration of promethazine is shown on the horizontal axis. The threshold for haemolysis of cells by promethazine (approximately $1{\cdot}25 \times 10^{-3}$ M) set an upper limit on the concentration used. The percentage of cells agglutinated is plotted on the vertical axis.

The inhibition of agglutination produced by promethazine ($1{\cdot}25 \times 10^{-3}$ M) was not influenced by the group of the red cells used. Thus in 6 samples with Group A cells the mean haemoglobin content of the supernatant was 0·196 g/100 ml, whereas with 6 samples of Group B cells it was 0·184 g/100 ml. The difference was not significant (t-test: $t = 0{\cdot}16$, $P = 0{\cdot}8$).

Since the estimation of agglutination depended on the measurement of the haemoglobin value of cells remaining in suspension, it was important to ascertain whether promethazine affected the sedimentation rate of fresh cells. Accordingly, observations were made on suspensions of Group O cells with Group O serum, together with saline or promethazine solutions and these mixtures were allowed to stand for 30 to 75 min. No significant difference was detected between the sedimentation rates of cells in the various samples. This result agrees with that reported by Shohl & Schmidt (1959). Steinbuch (1953) found that promethazine decreased the sedimentation rate in fresh blood but accelerated it in blood stored for more than four days. The cells used in these experiments were from freshly drawn blood (less than 24 hr old).

The inhibitory effect of promethazine on agglutination appears to be in the nature of a delaying factor and not a complete inhibition, since in qualitative tests performed on a slide rather than in a tube the cells appeared to agglutinate normally after a prolonged period of incubation at room temperature (15–20°). It was noted that when the control samples exhibited a high degree of agglutination, the inhibitory effect of promethazine was more marked.

Acknowledgement. The author is greatly indebted to May and Baker Ltd. who generously supplied promethazine hydrochloride in prepared solution and purified powder form.

Serology Section,
Department of Pathology,
Royal Women's Hospital,
Melbourne, Australia.

BRIAN D. TAIT

April 3, 1968

References

Barrie, J. U. & Tait, B. D. (1967). *Lancet*, **2**, 260.
Biermé, S. & Biermé, R. (1967). *Ibid.*, **1**, 574.
Seeman, P. & Weinstein, J. (1966). *Biochem. Pharmac.*, **15**, 1737–1752.
Shohl, J. & Schmidt, P. J. (1959). *J. Lab. clin. Med.*, **54**, 42–48.
Steinbuch, M. (1953). *Rev. Hemat.*, **8**, 31.

Gas-liquid chromatography of imidazoline salts

SIR,—A gas-liquid chromatographic method for analysis of imidazolines in pharmaceutical preparations has recently been published by Boon & Sudds (1967). Their treatment included both antazoline and naphazoline in the group of five heterocyclics investigated. These two compounds may be conveniently analyzed by a procedure which offers the advantages of reduced tailing and inclusion of an internal standard.

A dual column flame ionization chromatograph was operated under the following conditions: flash heater at 250°, column temperature at 239°, isothermal; detector temperature at 265°; helium carrier gas at 40 p.s.i.g. and 70 cc/min; hydrogen at 60 cc/min; air at 300 cc/min; and a recorder range of 10 with attenuation of 32 for carbazole and naphazoline and 64 for antazoline. These recorder adjustments are stated for use of manual quantization. Results were obtained using an integrator. The columns consist of 4 ft of 6 mm O.D. U-shaped glass tubing containing Chrom-Q, 100–120 mesh, treated with 1·0% KOH in methanol and coated with 5·0% of Apiezon-L using methylene chloride as the solvent.

Procedure. Add an aliquot of sample containing approximately 4–40 mg each of naphazoline salt and antazoline salt to a separatory funnel. Make the solution basic with sodium hydroxide and extract immediately with chloroform. Combine the chloroform extracts and add a portion of the internal standard solution containing approximately 4 mg of carbazole in chloroform. Evaporate to approximately 2 ml and inject 0·5 to 1·2 μl of the solution. Calibrate the sample by comparing the ratio of the peak areas of each imidazoline to the peak area of the internal standard.

Alcon Laboratories, Inc.,
6201 S. Freeway,
Fort Worth, Texas, U.S.A.

JOSE MOLINA
R. D. POE

February 20, 1968

Reference

Boon, P. F. G. & Sudds, W. (1967). *J. Pharm. Pharmac.*, **19**, *Suppl.* 88S–92S.