# Metabolic effect of *p*-aminosalicylic acid on human erythrocytes

*p*-Aminosalicylic acid (PAS) induces haemolysis in glucose-6-phosphate dehydrogenase (G-6-PD)-deficient subjects (Szeinberg, Sheba & others, 1957).

We have found that, after *in vitro* incubation with PAS under experimental conditions similar to those of Beutler's glutathione stability test (Beutler, 1957), erythrocytes from G-6-PD-deficient subjects showed no decline of reduced glutathione (G-SH) concentration. Erythrocytes with normal G-6-PD activity washed and suspended in buffered saline also showed no decline of G-SH content, although both PAS concentration and incubation time were increased two-fold (Table 1). No methaemoglobin was formed in erythrocytes after incubation with PAS although methaemoglobin has been claimed to be an obligate intermediary in the biochemical sequence of drug-induced haemolysis (Allen & Jandl, 1961). There was also no Heinz body formation in erythrocytes after incubation with PAS, as happens with acetylphenylhydrazine (APH). Similar experiments with haemolysate prepared from erythrocytes with normal G-6-PD activity also showed no decline of G-SH content or the formation of methaemoglobin (Table 1). Hence, permeability is not the limiting factor.

Table 1. *G-SH and Methaemoglobin levels after incubation with PAS Na and APH.* Twice washed normal human erythrocytes were suspended in isotonic buffered saline (pH 7·4) to give a haematocrit of 50%. A haemolysate was prepared from washed normal erythrocytes by freezing and thawing. Erythrocyte suspension or haemolysate were incubated in the presence of various substances at 37° with continuous shaking for 4 h. G-SH was measured by the nitroprusside method (Beutler, 1957) and methaemoglobin spectrophotometrically (Brewer, Tarlov & Alving, 1960)

| Additions to erythrocyte suspension or haemolysate (mM) | G-SH (μmol/ml erythrocytes) | Methaemoglobin (% of total Hb) |
|---|---|---|
| Erythrocyte suspension | | |
| None .. .. .. .. | 1·80 | 0 |
| APH, 30 .. .. .. .. | 0·03 | 31 |
| APH, 30 and glucose, 25 .. | 1·95 | 29 |
| PAS Na, 30 .. .. .. | 1·90 | 0 |
| PAS Na, 60 .. .. .. | 1·90 | 0 |
| Haemolysate | | |
| None .. .. .. .. | 2·18 | 2·3 |
| APH, 30 .. .. .. .. | 0 | 51 |
| PAS Na, 60 .. .. .. | 2·19 | 2·3 |

The failure of PAS to oxidize intracellular G-SH and haemoglobin places the drug apart from other known haemolytic drugs such as APH, primaquine, nitrofurantoin, α- and β-napthoquinone and certain vitamin K derivatives which have been shown to oxidize G-SH of G-6PD-deficient erythrocytes (Beutler, 1959). Only, napthalene, like PAS, has no oxidative action (Beutler, 1959).

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# Changes of thirst threshold produced by chlorpromazine

The effect of chlorpromazine on the activity of the hypothalamohypophyseal antidiuretic system has been described by Kovacs, Kovacs & others (1957), Moses (1964) and Boris & Stevenson (1967). As this system is morphologically and functionally close to the hypothalamic thirst centre it could be supposed that chlorpromazine might also effect the mechanism of thirst.

We have investigated the effect of chlorpromazine on the osmotic reactivity of thirst mechanism. The experiments were made with 10 mongrel dogs, 13–22 kg, fasted for 18 h but with free access to water. The osmotic reactivity of the thirst mechanism was examined under control conditions and after intravenous infusion of chlorpromazine, and was measured as the thirst threshold in relation to osmotic stimuli (Wolf, 1950). A solution of saline (5%) was infused (6·4 ml/min) into the saphenous vein of a dog having free access to water and with freedom of movement. When the dog began to drink, the infusion was stopped, and it was assumed that the osmotic load induced by the infusion had reached the thirst threshold. Measurements of the volume of water ingested, the volume of urine produced, and quantity of sodium excreted during the infusion were taken.

The level of thirst threshold was expressed by the magnitude of the sodium load (i.e. the number of m-equiv of $Na^+$ in the infusion less the number of m-equiv of $Na^+$ excreted in urine) necessary to induce the drinking response. The cellular dehydration produced at the point of thirst threshold by infusion of hypertonic NaCl was also calculated. The total body water and the extracellular water were measured in each dog. The plasma $Na^+$ concentration was also measured and the total amount of $Na^+$ calculated. The amount of $Na^+$ and water in the infusion are known, hence the shift of water caused by hypertonic infusion and cellular dehydration inducing the drinking reaction could be calculated (threshold cellular dehydration). In each dog, control measurements of thirst threshold were checked 4–6 times.

Chlorpromazine, (Specia-Largactil) 0·22 mg/kg, dissolved in 1·4 ml of 0·9% saline, was given by intravenous infusion 1 h before the thirst threshold measurement. Total body water was measured using tritiated water (Chwalinski, Mikulski & Kossakowska, 1965), and extracellular water by using sodium thiocyanate. The sodium concentration was measured by the Zeiss III flame photometer.

In 9 dogs, chlorpromazine, 0·22 mg/kg, lowered the osmotic reactivity of the thirst mechanism. One dog treated with 0·44 mg/kg showed a higher thirst threshold. The difference between cellular threshold dehydration (%) in controls ($4 \pm 0{\cdot}5$) and after chlorpromazine infusion ($6{\cdot}7 + 0{\cdot}7$) was statistically significant ($P < 0{\cdot}1$).

The volume of water drunk under the threshold stimulus did not differ significantly from the controls. Diuresis increased in 8 dogs during chlorpromazine infusion in a range from 14–204% with an average of 10%. It remained unchanged in one dog and in another decreased by 13%.

The lowering of osmotic reactivity of the thirst mechanism by chlorpromazine may be connected with its direct action upon the central nervous system. A secondary effect of this drug on the thirst mechanism, which might well be due to