Comparative Hemolytic Activity of Mephenesin, Guaiacol Glycerol Ether and Methocarbamol *in vitro* and *in vivo*. (23240)

EDWARD B. TRUITT, JR.* AND RICHARD B. PATTERSON

A. H. Robins Co. Laboratory, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem.

Two impediments to intravenous use of mephenesin are its hemolytic effect and the large volumes of solution required(1-6.) Guaiacol glycerol ether [3-(o-methoxyphenoxy)-1,2-propanediol] (GG) has comparable muscle relaxant effects, an expectorant action and much weaker hemolytic properties (7-14). Methocarbamol [3-(o-methoxyphenoxy)-1,2-propanediol monocarbamate] (AH R-85) has similar properties of extended duration with adequate solubility for parenteral use(15).

This investigation details *in vitro* and *in vivo* comparisons between GG, AHR-85 and mephenesin.

Methods. In vitro. Hunter's method(16) for erythrocyte fragility was adapted to variations in drug concentration. Drugs were dissolved in 0.015 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.4) in 0.9% NaCl with the following increments and ranges of concentration: mephenesin, 0.02% (0.82 to 0.92%), GG, 0.2% (2.0 to 3.0%) and AHR-85, 0.1% (2.0 to 2.5%). Heparinized, rather than oxalated blood, was used. Measurements were made of oxyhemoglobin in a Beckman spectrophotometer at 540 mμ after standing 55 minutes (with intermittent shaking) in a 25°C water bath followed by 5 minutes of centrifugation. Per cent hemolysis at each concentration was calculated by the formula:

$$\% \text{ Hemolysis} = \frac{\text{Opt. density of sample}}{\text{Opt. density of 100\% hemolysis}} \times 100.$$

The concentration of each drug producing 50% hemolysis (HC_{50}) was interpolated visually from a probit of % hemolysis versus log of concentration graph. This probit transformation of % hemolysis values seemed valid since Ponder(17) has shown the log concentration curves to be essentially sigmoidal except for a small amount of skewness near 100% caused by highly resistant erythrocytes.

Results. In vitro. The results obtained are set forth in Table I along with the calculated relative hemolytic activity of GG and AHR-85 compared to mephenesin as 100%. It can be seen that there is a small amount of variability in the concentrations required for the HC_{50} in different individuals, but the ratios between drugs for the same persons' erythrocytes are very uniform. The averages of these values indicate that at equihemolytic concentrations GG is about 31% and AHR-85 is close to 39% as hemolytic compared to mephenesin as a standard.

Methods. In vivo. The methods used were the van den Bergh test for total serum bilirubin and the method of Hunter *et al.* (18) for serum hemochromogen. Five unanesthetized dogs were used for 11 experiments using doses of 100 mg/kg of mephenesin and equimolar doses of GG (109 mg/kg) and AHR-85 (133 mg/kg). The drug solutions were injected intravenously at a rate of approximately 15 cc/min. after prior warming to 37°C. The concentrations used were mephenesin—2% (as Tolserol - Squibb, a supersaturated solution), AHR-85 - 3 and 4% (as aqueous solutions, the latter is saturated at 37°C) and GG - 4 and 5% (as aqueous solutions, the latter is saturated at 37°C). Venous blood samples were taken with a dry siliconized syringe and needle for a control, 30 minute, 1 hour and 2 hour samples. Serum bilirubin differences were taken between the control sample and the 30 minute, 1 hour and 2 hour samples for 5 control dogs. Student's t value was calculated for the bilirubin changes in drug experiments in comparison to the mean of these control differences and their standard devi-

* Present address: University of Maryland School of Medicine, Baltimore.

TABLE I. Concentrations of Mephenesin, GG and AHR-85 Producing 50% Hemolysis of Human Venous Blood in 1 Hr (HC_{50}) and Relative Activity between Drugs.

Donor	EBT	AMM	WCB	RBP	LAW	JC	Avg
			50% hemolytic conc., %				
Mephenesin	.88 .88	.85 .84	.90	.88 .89	.885	.87	.875
GG	2.84	2.73	2.93	2.92	2.88	2.82	2.85
AHR-85	2.22	2.16	2.34	2.29	2.26	2.27	2.26
			Relative hemolytic activity, % (mephenesin = 100%)				
Mephenesin / GG	31	31	30.7	30.3	30.7	30.8	30.75
Mephenesin / AHR-85	39.7	39.1	38.5	38.6	39.2	38.3	38.9

ation at comparable times by the formula:

$$t = \frac{\overline{X}_{Cont.} - X_{Exp.}}{S.D._{\overline{X}_{Cont.}}}.$$

Results. In vivo. The changes in serum bilirubin from the dogs' own controls are plotted in Fig. 1. Significant differences ($p \leqq 0.05$) are indicated by an "s." It may be seen from the graphs that the elevations of serum bilirubin above control values were not large in any instance. Intravenous infusion of 2% mephenesin caused a rise in serum bilirubin which was in most cases intermediate between that of the stronger and weaker solutions of GG and AHR-85 used.

With the serum hemochromogen test it was found that measureable hemolysis occasionally occurred in control samples. Thus it was not possible in all instances to determine whether the total amount of hemolysis was attributable to the drug alone or was caused in part by mechanical trauma to the blood

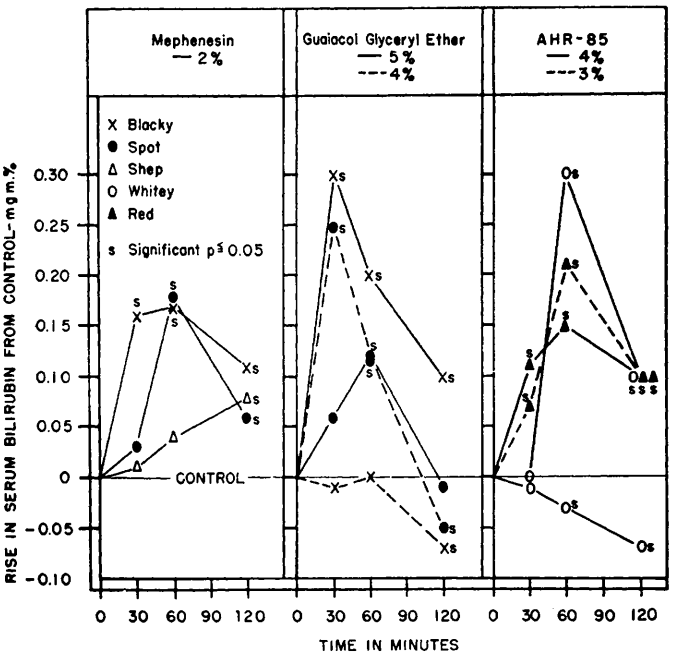

FIG. 1. Changes in serum bilirubin from control values in dogs after rapid intrav. inj. of aqueous solutions of mephenesin, GG and AHR-85. See text for explanation of test of significance.

cells during the handling of the blood. However, in no instance after infusion of a drug did the hemochromogen value exceed 88 mg/100 cc. This value, based upon a normal dog hemoglobin of 14 g/100 cc would represent only 0.63% of the total circulating hemoglobin.

Discussion. Ginzel(7) has stressed the importance of evaluating the therapeutic activity of mephenesin-like compounds in ratio to their hemolytic effect which limts their usefulness by the intravenous route. By this comparison GG, which has only slightly less anti-convulsant action than mephenesin, shows a decided advantage over mephenesin by the parenteral route. AHR-85, though somewhat less active immediately than mephenesin, shows marked advantage when the longer duration of action is considered(15).

These data *in vitro* and *in vivo* suggest that both GG and AHR-85 can be given intravenously in large doses (equivalent to 7 g of mephenesin I.V. in a 70 kg man) with little danger of an appreciable hemolytic reaction. The maximal value observed for the serum bilirubin was 0.4 mg%. This is only slightly above the normal human range (0.1 to 0.25 mg%). The maximum observed serum hemochromogen value did not exceed the human renal threshold for hemoglobin (100 mg%)(19) and represents hemolytic liberation of less than 1% of the normal total hemoglobin. The ratio of hemolytic activity *in vitro* between mephenesin and GG which Ginzel *et al.*(8) reported was based on total (100%) hemolysis and a 24 hour test. His finding of a 4:1 ratio of hemolytic activity favorable to GG is comparable to our *in vitro* value determined by HC_{50}'s at 1 hour and 25°C. Berger *et al.*(14) found this ratio to be 3.68:1 when comparisons of 75% hemolytic concentrations were made after 100 minutes. No comparable basis exists for comparing the *in vivo* results reported herein with other hemolytic data on mephenesin *in vivo* (5,6).

Summary. Mephenesin, guaiacol glyceryl ether (GG) and methocarbamol (AHR-85) have been compared as to their relative hemolytic activity *in vitro* and *in vivo*. Evidence is presented that GG and AHR-85 may be administered slowly as saturated aqueous solutions in large doses with little danger of an appreciable degree of hemolysis as indicated by the serum bilirubin and hemochromogen tests.

The authors would like to acknowledge the helpful advice of Dr. J. Maxwell Little.

1. *New and Non-Official Remedies,* J. B. Lippincott Co., Philadelphia, 1954.

2. Berger, F. M., *Pharmacol. Rev.,* 1949, v1, 243; in *J. Pharmacol. Exp. Therap.,* 1949, v96, pt.2.

3. Clendon, D. R. T., and Penfold, J. B., *Lancet,* 1949, v2, 987.

4. Griffith, H. R., Stephen, C. R., Cullen, W. G., and Bourne, W., *Anesthesiology,* 1949, v10, 61.

5. Wyngaarden, J. B., Tieche, H. L., and Seevers, M H., *ibid.,* 1949, v10, 529.

6. Pugh, J. I., and Enderby, G. E. H., *Lancet,* 1947, v2, 387.

7. Ginzel, K. H., *Arch. Exp. Path. u. Pharmakol.,* 1951, v212, 331.

8. Ginzel, K. H., Leupold-Lowenthal, H., and Weis, W., *Wein. med. Wschr.,* 1949, v99, 299.

9. Ginzel, K. H., and Wayand, E., *Anest. et Analg.,* 1952, v9, 14.

10. Smith, H. W., *J. Pharmacol. Exp. Therap.,* 1947, v91, 93.

11. Carrara, G., and Chiancare, F. M., *Boll. soc. Ital. Biol. Sper.,* 1947, v23, 1279.

12. Cycha, F. P., *Med. Monatsschr.,* 1952, v6, 166.

13. ———, *ibid.,* 1952, v6, 214.

14. Berger, F. M., Hubbard, C. V., and Ludwig, B. J., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 532.

15. Little, J. M., and Truitt, E. B., Jr., *J. Pharmacol. Exp. Therap.,* 1957, v119, 161.

16. Hunter, F. T., *J. Clin. Invest.,* 1940, v19, 691.

17. Ponder, E., *Hemolysis and Related Phenomena,* Grune & Stratton, New York, 1948.

18. Hunter, F. T., Grove-Rasmussen, M., and Soutter, L., *Am. J. Clin. Path.,* 1950, v20, 429.

19. Monke, J. V., and Yuile, C. L., *J. Exp. Med.,* 1940, v72, 149.

Received January 3, 1957. P.S.E.B.M., 1957, v95.