of the authors indebted to Dr. C. P. Leblond of McGill University for his stimulating influence in this field of endeavor.

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Received March 29, 1954. P.S.E.B.M., 1954, v85.

Effect of Propylene Glycol on Methemoglobin-Forming Capacity of p-Chloroacetanilide in Rat and Guinea Pig.* (20985)

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Propylene glycol has been used extensively as a non-toxic and metabolically inert solvent for the oral or parenteral administration of various water-insoluble compounds to animals and man. Studies concerning gastric secretion in rats(1), however, have shown rapid secretion by the stomach of Toluidine Blue when injected in aqueous solution, but no secretion when injected in propylene glycol solution. The supposedly innocuous nature of propylene glycol has also been disputed: a marked hypertensive effect in cats, rats, and rabbits(2), and LD_{50} doses in rats of 13 ml/kilo intraperitoneally and 28 ml/kilo orally(3) have been reported. In view of these findings, the effect of p-chloroacetanilide on the blood of rats and guinea pigs in presence and absence of propylene glycol was investigated.

Methods. Adult male Wistar rats, 200-400 g, and adult female guinea pigs, 500-630 g, were used. All animals received tap water and food *ad libitum*. The rats were maintained on Purina Laboratory Chow pellets and the guinea pigs on Purina Rabbit Laboratory Chow pellets. The test doses (Table I) were administered by intraperitoneal injection. p-Chloroacetanilide, only slightly soluble in water, was suspended in a finely divided state in 0.1% methylcellulose solution; the methylcellulose being used for its wetting property. Two hours after injection, 2 ml blood samples were obtained by heart puncture under light ether anesthesia. Heparinized syringes were used to prevent blood coagulation (3 mg sodium heparin/ml distilled water). Cell volume to plasma volume ratios were determined by the hematocrit method of Wintrobe and Lansberg(4); total blood pigment by the method of Sahli(5); and methemoglobin by the method of Michel and Harris(6).

Results. The results are summarized in Table I. A dual effect on the blood is evident. The injection of propylene glycol produced an increase in hematocrit over the normal value. There was a corresponding increase in total blood pigment which indicated loss of fluid from the blood. This increase in hematocrit was uniform in both species and was not influenced by the presence of p-chloroacetanilide. On the other hand, p-chloroacetanilide produced methemoglobinemia in both species in presence and absence of propylene glycol. The methemoglobin-producing effect of a propylene glycol solution of p-chloroacetanilide, was greater in the rat (8.09%) than in

* Supported by contract with the U. S. Atomic Energy Commission, Division of Biology and Medicine.

TABLE 1. Effect of 100 mg/Kilo p-Chloroacetanilide and 4 ml/Kilo Propylene Glycol on Blood of Rat and Guinea Pig.

Group and treatment*	No. animals		Hematocrit,† %		Total pigment, g %		Methemoglobin, % of total pigment	
	Rat	Guinea pig	Rat	Guinea pig	Rat	Guinea pig	Rat	Guinea pig
1. 100 mg/kg pCAA in 4 ml/kg PG	9	4	55.7 ± 3.66	50.4 ± 2.64	19.8 ± 1.82	18.5 ± .42	8.09 ± 3.76	4.21 ± .06
2. 4 ml/kg PG	7	4	51.0 ± 13.45	51.8 ± 5.68	20.2 ± .36	18.4 ± 2.41	1.51 ± .36	2.21 ± .04
3. 100 mg/kg pCAA in 4 ml/kg MC	10	4	45.4 ± 2.47	43.8 ± 2.63	16.9 ± .41	16.7 ± 1.49	40.8 ± 15.78	6.89 ± .09
4. 4 ml/kg MC	3		46.3 ± 5.30		17.4 ± .40		1.75 ± .07	

* pCAA = p-Chloroacetanilide; PG = propylene glycol; MC = 0.1% aqueous methylcellulose solution.
† Comparison of totals of groups 1 and 2 with 3 within each species was statistically significant. $p < 0.02$. Normal hematocrits are 45% for the guinea pig(7) and 42% for the rat(8).

the guinea pig (4.21%); and when administered in aqueous suspension, the methemoglobinemia greatly increased and the species difference was accentuated (rat, 40.8%; guinea pig, 6.89%).

All rats receiving propylene glycol alone became markedly apathetic and showed ataxia, particularly of the hind legs. Only one of 4 guinea pigs showed slight ataxia. The ataxia is possibly caused by lactic acid accumulation in the muscles, more evident in the active rat than in the more docile guinea pig. The increased viscosity of the blood could reduce its oxygen-carrying capacity enough to cause oxygen debt in the muscles. Furthermore, since propylene glycol has been shown to exhibit a glycogenic effect(9), it may competitively inhibit the reconversion of muscle lactic acid to glycogen. Propylene glycol also has been shown to be converted to lactic acid *in vitro*(10). All animals receiving p-chloroacetanilide in propylene glycol were ataxic. Most of the rats receiving the p-chloroacetanilide suspension became cyanotic and prostrate. The guinea pigs receiving the suspension were ataxic but not cyanotic, which is understandable since the methemoglobin did not reach the level (18-20%) necessary for observable cyanosis(11).

The reason for the modification of the methemoglobin-producing effect of p-chloroacetanilide by propylene glycol is not clear. The propylene glycol may reduce the rate of absorption and thus the availability of the p-chloroacetanilide at any one time; yet, distribution studies on the Cl^{36} labeled compound have shown ready absorption(12). Conversely, the propylene glycol may act as a biochemical poison on the enzyme mechanisms necessary to produce the methemoglobin-forming metabolite of the p-chloroacetanilide. It is evident in any case, however, that propylene glycol should be avoided whenever possible as a solvent for biological work, and that results attributed to compounds administered in propylene glycol solution should be carefully interpreted since the propylene glycol may alter the physiological response.

Summary. Rats and guinea pigs injected with propylene glycol, 4 ml/kg, with or without p-chloroacetanilide, showed a uniform in-

crease in hematocrit to 51% as compared with a normal value of 42-45%. Animals receiving no propylene glycol showed no increase in hematocrit above the normal value. p-Chloroacetanilide, 100 mg/kg, dissolved in propylene glycol, 4 ml/kg, produced methemoglobinemia in both species; to a greater extent in the rat than in the guinea pig: 8.1% and 4.2% of the total blood pigment, respectively. Propylene glycol alone, 4 ml/kg, produced no increase in methemoglobin above normal range: 1.5% for rats, and 2.2% for guinea pigs. p-Chloroacetanilide, 100 mg/kg, in aqueous suspension increased the methemoglobinemia in both species; the increase more pronounced in the rat than in the guinea pig: 40.8% and 6.9% of the total blood pigment, respectively. These results indicate a decrease in production of the methemoglobin-producing metabolite of p-chloroacetanilide in the presence of propylene glycol. It is suggested that results attributed to a compound administered in propylene glycol solution should be carefully interpreted since the propylene glycol may make a marked contribution to the physiological activity of the mixture.

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Received March 8, 1954. P.S.E.B.M., 1954, v85.

Synovial Fluid. I. Comparison of Sodium and Potassium Concentrations in Normal and Diseased Joint Fluid.* (20986)

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The finding of excess synovial fluid in various disease states has prompted us to investigate the sodium and potassium concentrations of fluids obtained from arthritic joints and compare them with those obtained from the normal. Previous investigations on synovial fluid have utilized material obtained from cattle(1,2), horses(3), dogs(4), edema effusion(5), and human autopsy material (2,6,7).

Material and methods. The synovial fluid samples were aspirated from the knee joints of 10 normal living subjects, 10 autopsy cases, 25 patients with effusions in untreated acute rheumatoid arthritis, and 5 individuals with a diagnosis of osteoarthritis with effusion. After repeated failure to obtain fluid from living subjects, it was found that by allowing the subjects to remain inactive for 8-12 hours prior to the procedure, volumes of fluid up to one cc could be obtained from the knee joint with only moderate difficulty. A 20-gauge needle was introduced under the medial border of the patella directly into the joint cavity. Prior infiltration of the skin with 1% procaine made the procedure relatively painless. The

* Supported by grants from the Am. Med. Assn., Chicago, Ill., and the National Institutes of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service, Bethesda, Md.

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