pression of the disease process. Although curative treatment with Sanguinin gave only insignificant results, the survival percentages increased approximately 6-fold over the controls when the infection was preceded even by one single dose of Sanguinin (series 2, Table I). It seems, therefore, that the pretreatment is the decisive factor influencing the course of the streptococcal infection. Whereas, it is improbable that the suppression of the infection is due to a direct action of Sanguinin on the agent, it appears that the mode of action is indirectly eliciting the body defense mechanism by a non-specific stimulation. The newly established property of Sanguinin as a powerful enzymatic inhibitor(6) deserves attention in the further analysis and interpretation of its antibacterial activities.

Summary and conclusions. The blood hydrolysate Sanguinin was tested *in vivo* in 268 albino mice accompanied by 221 untreated controls for its effectiveness against the beta-hemolytic *Streptococcus zooepidemicus*. Sanguinin solution (20 mg/ml) was inoculated subcutaneously (0.25 to 1.0 mg/mouse daily, usually for 4-6 days) either prior or after infection. Suppressive effect on the course of the infection was noted in all series of treated mice. A significant increase in survival time was noted in the pretreated series with an average survival percentage of 38.9 compared with 6.0 of the total of untreated controls. It does not appear that a direct correlation can be established between the survival rates and the intake of Sanguinin or the length of its administration, however, pretreatment seems to be of decisive value.

The authors are greatly indebted to Dr. J. G. Bieri, Department of Biochemistry and Nutrition, for his valuable advice during the course of this study.

Received September 19, 1951. P.S.E.B.M., 1951, v78.

Pharmacological Properties of a New Parasympathetic Blocking Agent, N,N Dimethyl 4-Piperidylidene 1,1 Diphenylmethane Methyl Sulfate (Prantal) (19145)

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The investigations of Acheson and Moe(1), Longino, Grimson *et al.*(2,3) and Hambourger, *et al.*(4) have stimulated the search for new substances capable of blocking synaptic transmission. Interest has been furthered by encouraging clinical results reported for parasympathetic blocking agents in the management of peptic ulcer(5,6). During the study in this laboratory of a series of new quaternary salts, several compounds were found to have autonomic ganglion blocking properties. One of these, N,N dimethyl 4-piperidylidene 1,1 diphenylmethane methyl sulfate (Prantal) (7), exhibits some unusual pharmacodynamic properties which distinguish it from previously known orally active atropine-like drugs. Pharmacological data and preliminary clini-

* The authors are indebted to S. W. Lee for valuable suggestions, and to S. Neuwirth for the biometric evaluation of data.

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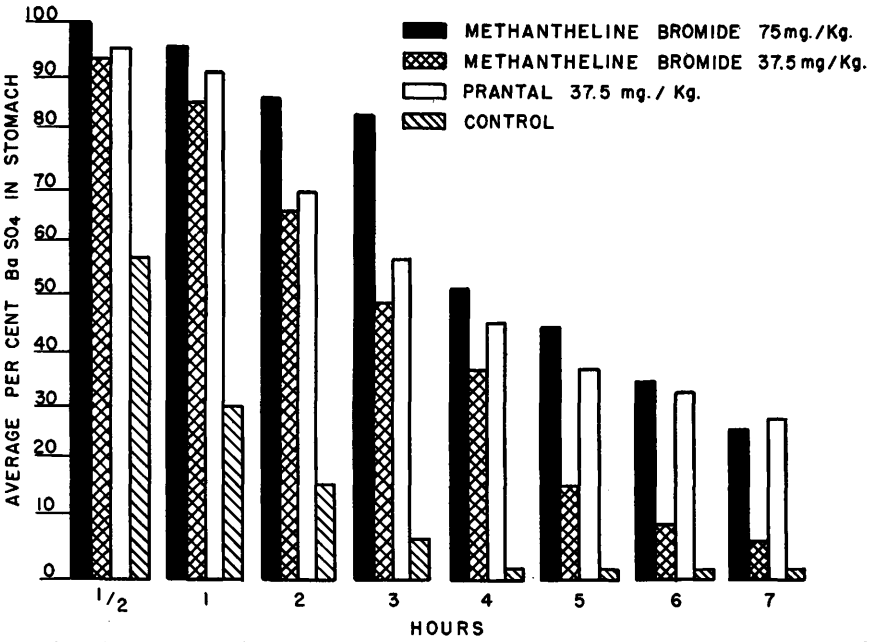

FIG. 1. Gastric emptying time following oral administration of Prantal to 4 dogs as determined by roentgenological examination after a barium sulfate meal.

cal evidence suggest that Prantal primarily acts upon the portion of the parasympathetic nervous system associated with gastric secretion and motility. In doses which elicit this selective parasympathetic blocking action, it produces no mydriasis in animals, and rarely, mydriasis or xerostomia in man.

Parasympathetic blocking action in dogs. Parasympathetic blocking effect was measured in dogs by determining the amount of Prantal required to block cardiac arrest induced by faradic stimulation of the divided vagus nerve. The cardiac arrest was completely inhibited by 0.05 mg/kg of Prantal or 0.05 mg/kg of methantheline bromide given intravenously. The methantheline bromide value is in agreement with that reported by Longino, Grimson, *et al.*(2). Both substances act for 10 to 15 minutes with this low dose, and with increasing doses the action is prolonged. *Sympathetic blocking action.* The sympathetic ganglion blocking action of Prantal was evidenced by (1) inhibition of the carotid sinus pressor reflex (increase in blood pressure during occlusion of both common carotid arteries in the dog, and (2) the block of faradic stimulation of the isolated superior cervical ganglion in the cat. The compound was injected intravenously into the femoral vein. Prantal partially or completely blocked the carotid sinus reflex in dogs when 2 to 5 mg/kg of the drug were given. Partial or complete blockade of the superior cervical ganglion in the cat also occurred with 2 to 5 mg/kg. The intravenous dose capable of blocking the sympathetic nervous system was 50 to 100 times the dose required to produce parasympathetic blockade.

Gastric emptying time in dogs. Experiments were performed with 4 trained, non-anesthetized dogs employing a technic similar to that described by Longino, Grimson, *et al.*(2). Prantal was fed in capsules at a dose of 37.5 mg/kg 75 minutes before 30 g of barium sulfate in 100 ml of water were administered by stomach tube. With the intragastric feeding of barium sulfate at "zero" time, roentgenograms were taken at ½, 1, 2, 3, 4, 5, 6, and 24 hours. Prantal was highly effective in delaying the movement of barium sulfate from the stomach; approximately 60% of the barium sulfate was still present in the stomach after 3 hours; approximately 40% after 5 hours. In control trials

(no medication). barium sulfate rapidly entered the duodenum; after one-half hour approximately 60% of the barium sulfate remained in the stomach, and only 5% after 3 hours. The average of the estimates of the residual barium sulfate present in the stomach at various time intervals, as shown in Fig. 1, suggest that Prantal in these tests is more effective and longer acting than methantheline bromide.

Inhibition of gastric secretion in dogs. The effect of Prantal and methantheline bromide was studied on the gastric secretion of dogs anesthetized with chloral hydrate. Each compound was tested in 4 dogs. The gastric fluid was collected by means of a cannula passed through a duodenal incision and anchored into the pyloric orifice. Copious gastric secretion was induced by the continuous parenteral infusion of histamine dihydrochloride at the rate of 0.12 mg/kg/hour. The antisecretory action was evaluated by comparing gastric juice flow collected before and after the injection of drug. Collection of secretion was continued until the drug inhibitory effect disappeared. After the intravenous injection of 1.0 mg/kg of Prantal, the per cent reductions in gastric secretory volume for the individual dogs were 50.0%, 90.0%, 72.0%, and 53.6% (mean of 66.4%); the reductions in the total acid were 66.7%, 88.2%, 97.7%, and 44.1% (mean of 74.2%). In the dogs given 1.0 mg/kg of methantheline bromide intravenously, the per cent reductions in gastric juice flow were 88.9%, 54.5%, 71.9%, and 88.1% (mean of 75.9%); reductions in total acid were 98.0%, 60.0%, 58.3%, 86.7% (mean of 75.8%). Prantal and methantheline bromide did not differ in activity in these experiments.

Mydriatic activity. Solutions containing 0.1%, 1%, and 2% of Prantal were applied to the conjunctival sac of rabbits for a period of one minute (6 or more animals per dilution). Pupillary dilatation and impaired sensitivity to changes in light intensity occurred with the 2.0% dilution. Methantheline bromide was applied to the rabbit eye at 0.1% and 0.5% concentrations and both solutions produced mydriasis; the 0.5% dilution caused mydriasis lasting for 2 to 3 hours. The concentrations which produced mydriasis are similar to those reported by Hambourger, *et al.*(4) for methantheline bromide. In other experiments, the compounds were compared in rabbits by intravenous injection, and 1.0 mg/kg of methantheline bromide caused mydriasis in 2/20; at 2.0 mg/kg in 6/6. No mydriasis followed the intravenous administration of 2.0, 4.0, or 8.0 mg/kg of Prantal. Further evidence of the absence of mydriasis after Prantal was obtained in dog experiments described above (Fig. 1) at doses which inhibit gastric motility for several hours. In these tests, methantheline bromide induced mydriasis which persisted for 1 to 2 hours.

Specificity. The specificity of Prantal in its parasympatholytic action is indicated by several observations. The low mydriatic properties in rabbits and in dogs were mentioned above. During the x-ray studies with dogs, it was observed that Prantal exhibited little delaying action upon the transit time of a barium sulfate meal through the small intestine although stomach emptying time was greatly delayed. In anti-acetylcholine experiments *in vitro* (modified Magnus-Dale technic) the median effective concentration for Prantal was 10.75 μg/liter, and 1.25 μg/liter for atropine sulfate. These dilutions caused a 50% inhibition of maximal spasm of guinea pig ileum induced by 20.0 μg/liter of acetylcholine chloride. The intravenous injection of 0.05 mg/kg of methantheline bromide inhibited the vasodepressor response in dogs to intravenously administered acetylcholine chloride (0.1 mg/kg); whereas, 0.4 mg/kg of Prantal was required to obtain the same degree of inhibition. In the previously cited experiments demonstrating the ability of Prantal (0.05 mg/kg) to block cardiac arrest induced by faradic stimulation of the divided vagus nerve, Prantal and methantheline bromide did not differ in activity. *In vivo,* orally administered Prantal prevented bronchospasm and death in guinea pigs caused by the intravenous injection of an otherwise lethal dose of acetylcholine chloride (14 mg/kg). The median effective dose for Prantal was 27 mg/kg; for oral methantheline bromide, 62 mg/kg. The tremors, convulsions, and death induced in mice by subcutaneous injection of 2.0 mg/kg

TABLE I. Oral Acute Toxicity of Prantal in Rodents.

Species	No. of animals	LD_{50}, mg/kg	Confidence limits (P = .05), mg/kg
Mouse (Manor)	64	317	269 to 361
Rat (Rockland)	30	1107	900 to 1362
Guinea pig (Manor)	18	404	254 to 642

of physostigmine were antagonized by subcutaneous atropine sulfate (ED50 5.0 mg/kg); the subcutaneous ED50 for Prantal was 85 mg/kg, and for methantheline bromide, 13 mg/kg. Antihistaminic experiments *in vitro*, and tests *in vivo* with guinea pigs did not demonstrate any significant antihistaminic activity for Prantal.

Acute toxicity in rodents. The oral acute toxicity for Prantal was determined in the mouse, rat, and guinea pig, and values are listed in Table I. Graded doses of aqueous preparations were fed by stomach tube or oral syringe to groups of 6 or more animals. Mortality, over a 5-day period, was recorded, and the median lethal dose (LD50) with confidence limits was computed by the method of Litchfield and Wilcoxon(8). The intraperitoneal median lethal dose (LD50) in 18 to 22 g non-fasted mice was 47 mg/kg. In mice, rats or guinea pigs death was usually caused by respiratory depression and collapse.

Acute intravenous toxicity in dogs (infusion). Mongrel dogs were anesthetized with chloral hydrate or sodium pentobarbital, and the carotid blood pressure, pulse and respiration rate were recorded. Prantal was infused at an average rate of 0.280 mg/kg/min into the right femoral vein of 5 dogs weighing 4.1 to 6.0 kg; a mean lethal dose of 41.6 mg/kg was found. No significant change in the respiration rate or blood pressure was seen until just prior to death; pulse rate decreased steadily until death. Death was due to respiratory failure. Intravenous infusion of methantheline bromide into 4 dogs weighing 8.0 to 9.0 kg at an average rate of 0.300 mg/kg/min caused a distinct drop in blood pressure with the onset of the infusion, and this continued until death. A drop in the pulse rate was seen; no significant change in the respiration rate occurred until just prior to death. The mean lethal dose for methantheline bromide was 22.7 mg/kg. These data indicate Prantal to be approximately ½ as toxic as methantheline bromide by intravenous infusion in the dog.

Safety. Acute toxicity data for several species indicate a high safety ratio between toxic and parasympatholytic dosages. In acute intravenous studies with dogs, Prantal was less vasodepressor than atropine sulfate, or methantheline bromide. Subacute toxicity tests in mice, chronic experiments in rats, and detailed chronic studies with large over-dosages in dogs have not shown cumulative toxicity. During these investigations, growth rate and hematological findings were normal. At autopsy, no gross pathological changes were seen, and histopathological study of various organs disclosed no toxic effects.

Antidote. Experiments conducted in dogs with intramuscular or intravenous Prantal overdosage showed that the first sign of intoxication was tachycardia, followed by mydriasis, central nervous system depression, and respiratory depression. In tests with dogs, physostigmine intramuscularly (1 mg/kg) was found to be a satisfactory antidote when used even after the onset of respiratory depression. Supportive artificial respiration was also applied when warranted.

The parasympatholytic activity and low toxicity in experimental animals appeared sufficiently encouraging to warrant evaluation of Prantal in man. Preliminary results of oral or parenteral administration of test doses in 24 patients with peptic ulcer and of continuing treatment in 17 indicate therapeutic usefulness and minimal side effects (Grimson, Flowe, Rowe, and Emlet)(9). Gastric motility is inhibited an hour and longer following 50 mg intramuscularly or 100 mg orally. Gastric secretions are usually reduced in volume with marked lowering of total and free hydrochloric acid titers following 50 mg intramuscularly. Reduction is variable after a

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single oral dose. Continuous oral treatment using 100 mg(9) or 50 to 100 mg(10) every 4 or 6 hours usually relieves symptoms of ulcer.

Summary. (1) A new chemical compound has been found which has high oral and parenteral parasympathetic blocking activity. This compound, N,N dimethyl 4-piperidylidene 1,1 diphenylmethane methyl sulfate (Prantal), is unique in its specificity of action within the parasympathetic system, *i.e.*, it primarily inhibits gastric motility and gastric secretion. The intravenous sympathetic ganglion blocking dose is 50 to 100 times that required for intravenous parasympathetic blockade. (2) In dogs, roentgenologic observations of the movement of barium sulfate from the stomach indicate that orally administered Prantal delays gastric emptying time more effectively, and is longer acting than methantheline bromide. Additional dog experiments showed the ability of Prantal to reduce the volume and titratable total acid of gastric secretions. (3) After oral medication in dogs, Prantal produced no mydriasis at doses which inhibit gastric motility for several hours, whereas methantheline bromide caused mydriasis which persisted for one to 3 hours. In rabbit tests by topical application to the eye, mydriasis occurred with a 0.1% concentration of methantheline bromide, whereas 1.0% of Prantal was not mydriatic; mydriasis occurred at the 2.0% concentration of Prantal. By intravenous injection in rabbits methantheline bromide caused mydriasis at 1.0 and 2.0 mg/kg doses; no mydriasis occurred at 2.0, 4.0 or 8.0 mg/kg doses of Prantal.

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Received August 13, 1951. P.S.E.B.M., 1951, v78.

Regulation of Breathing in Man at Altitude.* (19146)

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Peracute hypoxia occurs in an aviator at high altitude if he loses his oxygen supply or if he suffers sudden decompression. The time that he will retain consciousness depends upon several factors, some of which have been reported(1). It has been shown that the addition of carbon dioxide to the inspired air increases the consciousness time of man at altitude(2). This investigation has been extended for the purpose of studying the relative influence of hypoxic and carbon dioxide stimuli upon pulmonary ventilation in the peracute hypoxic state. A simulated altitude of 25,000 feet was selected for the reason that in previous tests it was found that this altitude was somewhat marginal in its effect upon consciousness, the loss of which occurs only after several minutes. This allows sufficient time to record pulmonary ventilation for a few minutes and permits any respiratory change to reach an approximate steady state. Thus the pattern of the rapid hypoxia response can be more clearly observed.

Procedure. Ten experimental subjects were used in this investigation. They were medical students, physically fit and in good health. All had previous experience in the low pressure chamber during exposures to simulated high altitude. From 4 to 6 separate tests were made on each subject. Pulmonary ventilation was determined from photokymographic records as previously described(1). Thus a continuous record of pulmonary ventilation was obtained. Standard Air Force A-13 oxygen masks were used. These were fitted to prevent leakage and a leakage test was

* Work done under contract with the Aero Medical Laboratory, Air Research and Development Command, U.S.A.F.

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