with fluorescent antibody were investigated and modifications introduced into the method that should make it as sensitive and specific as the procedure using neutralization tests. Identification of the viruses was made much more rapidly with fluorescent antibody.

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Absence of a Renal Effect from Two Substituted Propanediols: Meprobamate and Mebutamate. (28615)

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Substituted propanediols are widely used in medicine as skeletal muscle relaxants and psychotropic agents. Recently, mebutamate (2-methyl-2 sec-butyl-1,3-propanediol dicarbamate) was introduced as an antihypertensive agent. Published data indicate that the primary site of action of this drug is the central nervous system(1): however, urine electrolyte excretion was not studied, and the possibility of renal participation in this hypotensive effect was not excluded.

Perhaps the most commonly used drug of this group is meprobamate, (2-methyl-2-n-propyl 1,3-propanediol dicarbamate). This drug has also been reported to have weak hypotensive activity(2). It was the purpose of this investigation to determine if a renal action, either on electrolytes or hemodynamics, could be demonstrated for these compounds in the dog. In these experiments renal plasma flow was only moderately depressed by mebutamate and electrolyte excretion was not affected by either drug.

Materials and methods. Conscious, trained, beagles of both sexes, raised in these laboratories were used. Food, but not water, was withheld for 24 hours prior to the experiment. All animals were given 20 ml/kg of tap water by stomach tube at start of experiment. Thirty minutes later the bladder was drained and washed with distilled water and this material was discarded. The subsequent urine samples were collected by a catheter which drained directly into cylinders containing toluene. The bladder was then washed twice with 10 ml of distilled water and this rinse was added to the urine sample. Further details of experiments are given in Table I.

Blood samples were taken in heparin-rinsed syringes at the mid-point of each urine collection period.

Drugs were supplied as the pure powder (Wallace Laboratories, Cranbury, N. J.) and given intravenously in a 20% solution of propylene glycol in saline. Concentration was adjusted so that each dose was given as 1 ml of the vehicle per kg of body weight. Due to the limited solubility of these compounds, the solutions of higher concentrations were warmed prior to administration to prevent precipitation.

Glomerular filtration rate (GFR) was measured by the clearance of inulin using the direct resorcinol method without alkali treatment, according to Schreiner's modification of the method of Roe, Epstein and Goldstein(3).

TABLE I. pH, Flow and Electrolyte Excretion Before and After High Intravenous Doses of Meprobamate and Mebutamate.

	Meprobamate, 90 mg/kg					Mebutamate, 50 mg/kg				
Time, min	Flow, ml/min	pH	Na^+	K^+	Cl^-	Flow, ml/min	pH	Na^+	K^+	Cl^-
			⌜—μeq/min—⌝					⌜—μeq/min—⌝		
− 90			Water: 20 ml/kg by stomach tube.							
− 60			Bladder drained and washed.							
− 30	.23	6.5	3	4	3	3.20	7.0	11	17	18
0*	.38	6.6	5	5	5	2.40	6.9	14	14	8
+ 30	.33	6.6	4	5	5	.76	6.9	13	6	15
+ 60†	.37	6.6	4	5	5	.45	7.4	6	6	18
+ 90	.33	6.5	6	6	3	.18	6.4	14	4	6
+180	.20	6.3	4	4	3	.20	6.2	9	6	10
+210	.02	6.3	2	2	2	.18	5.7	9	5	9
+240	.10	6.1	2	3	1					
+270	.12	6.0	1	3	2					

* Drugs given immediately after urine collection at time 0.
† Respiration stopped for short period after meprobamate. For details, see text.

Renal plasma flow (RPF) was measured by p-aminohippurate clearance using Smith's modification(4) of the method of Bratton and Marshall. Chlorides were determined by Brun's modification of the Schales and Schales method(4). A Baird Atomic flame photometer, model KY using a lithium internal standard, was used for determination of sodium and potassium. For pH, a Photovolt model 115 pH meter with a glass electrode was used. The measured pH was corrected to body temperature by subtracting 0.0014 pH units for each degree that room temperature was lower than 37°C.

Results. Meprobamate: A series of 5 experiments using male dogs was performed with this compound in which urine flow, pH, and electrolyte output were measured. The doses used were: 1, 3, 10, 30, and 90 mg/kg. Chlorides were determined for all samples, but sodium and potassium were only measured for the highest dose, since no change in chloride output was observed following lower doses. It was felt that any drug-related changes in the relative quantities of Na^+ and K^+ would be most pronounced in the latter experiment. However, no changes were detected in any of the electrolytes measured, regardless of the dose used. In some experiments the urine pH tended to fall with successive samples, but this change did not always occur, and when present, did not appear to be dose related. The protocol and results for the 90 mg/kg experiment are presented in Table I. The period of urine collection was extended to 6½ hours after drug, in order to detect any delayed effect which might appear. Approximately one hour after the drug was given, respiration ceased, but reflexes were still active. Following artificial respiration, spontaneous breathing returned and full recovery followed. A delayed onset of CNS effect has been reported previously (5) and was also seen in these experiments, but no latent renal effect was observed. It should be noted that what appears to be an antidiuretic effect of the drug in these and following experiments actually represents the end of the water diuresis and occurred in all experiments regardless of the dose or drug given.

Inulin and PAH clearances were determined in 2 female beagles using a dose of meprobamate of 30 mg/kg. In addition to these measurements, flow, pH, Na^+, K^+, and Cl^- were determined. The results of one experiment are presented in Table II. No significant changes were seen in either experiment. In the experiment of Table II the animal became extremely excited by administration of water by gavage and the initial high urinary pH probably is a reflection of the renal response to the hyperventilation seen at that time.

Mebutamate: Urinary electrolytes, pH, and flow were measured in male dogs following doses of 10, 30, and 50 mg/kg in separate experiments. As above, chloride was determined for all samples, and sodium and potassium for the highest dose only. Results of

TABLE II. Effect of Minimally Ataxic Doses of Meprobamate and Mebutamate on Renal Hemodynamics and Electrolyte Excretion.

	Meprobamate, 30 mg/kg							Mebutamate, 20 mg/kg						
Time	Flow, ml/min	pH	Na^+	K^+	Cl^-	Inulin clearance	PAH† clearance	Flow, ml/min	pH	Na^+	K^+	Cl^-	Inulin clearance	PAH† clearance
			μeq/min			ml/min				μeq/min			ml/min	
−120														
− 90														
						Began PAH and inulin infusion: water load 20 ml/kg.								
− 60	.84	8.0	17	57	30	58	176	1.00	5.7	7	7	10	26	138
− 30	1.44	7.6	10	29	20	52	160	1.40	5.9	5	8	5	25	120
0*	3.60	6.9	9	18	13	—	—	1.25	6.2	3	6	6	28	126
+30	2.70	6.6	7	11	9	55	145	.83	6.4	5	5	15	26	97
+60	1.03	6.9	15	22	5	54	153	.36	5.7	30	16	31	25	104
+90	.36	7.3	13	14	3	52	181	.50	5.8	8	12	31	28	102
+120	.96	6.9	18	13	32	51	160	.16	6.2	7	10	14	—	—

* Drug given immediately following urine collection at Time 0.
† p-Aminohippurate.

the latter experiment are contained in Table I. In this experiment, as in the lower doses, no change was seen after drug administration.

A dose of 20 mg/kg was selected for inulin and PAH clearances. Two experiments were performed and the results of one of these are presented in Table II. In both experiments, a slight drop in PAH clearance was seen. The slight increase in electrolyte excretion observed in this experiment was within the normal variation seen in these laboratories and was not considered to be a true drug effect.

Discussion. The data show that in the conscious dog, neither meprobamate nor mebutamate alters renal excretion of sodium, potassium or chloride in doses far exceeding those used clinically(6,7). An additional observation arising from these experiments is the persistence of control levels of electrolyte excretion following doses of these compounds which produced paralysis of the high limbs, and in one case, temporary respiratory arrest. Thus, while these compounds do not alter excretion of electrolytes, they are free from renal effects even in toxic doses.

For inulin and PAH clearance experiments, the smallest dose of each drug was selected which would produce some signs of ataxia. In both cases this dose fell well below the highest dose used in the electrolyte experiments, but presumably well above doses which might be given clinically. Berger, *et al.*(1) have reported a drop in renal blood flow and renal artery pressure in the anesthetized dog following the same dose (20 mg/kg) of mebutamate used in our experiments. However, the authors did not feel that this change was significant. It should be noted that the small drop in inulin clearance seen in our experiments was not accompanied by changes in electrolyte excretion. From the foregoing, it may be concluded that neither of these compounds could be expected to show any renal effect, either on hemodynamics or electrolyte excretion in situations where they might be used clinically. No evidence was obtained which indicates that either of these compounds has activity other than the central effects previously described.

Summary. To determine whether the hypotensive effects of meprobamate or mebuta-

mate could be related to a renal action, experiments were performed using the conscious, trained beagle in which urinary electrolytes and renal clearances were measured. Electrolytes were not affected by intravenous doses of 1-90 mg/kg of meprobamate or 10-50 mg/kg, of mebutamate. Clearance of inulin and p-aminohippurate were measured using doses of 30 mg/kg of meprobamate and 20 mg/kg of mebutamate. Neither clearance was affected by meprobamate, and there was only a small reduction in PAH clearance following mebutamate administration. It was concluded that these compounds are without significant renal effects.

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pH, Protein, and Semliki Forest Virus Infectivity.* (28616)

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An effect of pH and serum protein on the persistence of arbovirus infectivity has been known for many years(1,2). Loss of Semliki Forest virus (SFV) infectivity during attempts at purification led us to investigate the relationship between pH and stability of SFV in the presence and absence of protein. Persistence of SFV and Western equine encephalitis (WEE) virus infectivity in several tissue culture diluents was also studied and a possible stabilizing effect of amino acids noted.

Materials and methods. SFV and WEE were prepared as 10% W/V homogenates of infected mouse brain in sterile distilled water. After centrifugation for 20′ at 1500 *g* to sediment cellular debris, the supernate was frozen as 1 ml aliquots in sealed glass ampules and stored in a CO_2 (dry ice) box.

Crystalline bovine serum albumin purchased from Mann Research Laboratories had been prepared by electrophoretic separation in cellulose acetate. Tissue culture media were obtained from Microbiological Associates, Bethesda, Md.

The preparation of sodium phosphate buffer solutions, determination of pH, methods of dilution, incubation of serial dilutions, and mouse assay of infectivity were as described previously(3), with the exception that a thawed ampule of virus was immediately diluted 10-fold in chilled sterile distilled water to give a 10^{-2} dilution of infected mouse brain, instead of a 10^{-0} stock being diluted 100-fold. From the 10^{-2} dilution of virus, subsequent dilutions were made in one of the solutions under study using chilled diluents and glassware. One set of dilutions was immediately inoculated into groups of 5 male 18-24 g Swiss-Webster mice per dilution. The second set was incubated for 2 hours in a constant temperature water bath and then similarly assayed for mouse infectivity, as judged by lethality. SFV was assayed for infectivity by i.p. inoculation. WEE was assayed by i.c. inoculation because this strain was of low pathogenicity by other than i.c. injection. Mice were observed for 2 weeks

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