paralysis after administration of the virus (Table IV).

Summary. The influence of the level of 6 common minerals on the susceptibility of Swiss mice to Theiler's GDVII virus has been investigated in more than 1100 animals.

No demonstrable effect on the susceptibility to this infection was found by varying the level of calcium, magnesium or chlorine in the diet, some effect was noted with sodium, while striking results were obtained with potassium and phosphorus.

A progressively decreased resistance was observed as the amount of potassium or of phosphorus was increased in the diets up to the optimal levels.

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Influence of Various Pharmacologic Substances on the Emetic Effect of Intravenous Glutamic Acid in Dogs.

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The intravenous administration of amino acid mixtures of protein digests has become a promising method to aid in maintaining nitrogen balance in patients unable to take adequate food by mouth. A side effect, however, which imposes certain practical limitations is the occasional incidence of nausea and vomiting, particularly when the solutions are administered too rapidly. The chief offender was found to be glutamic acid (Madden *et al.*,[1] and Unna and Howe[2]). Aspartic acid has been shown to have a similar effect (Unna and Howe,[2] Madden *et al.*[3]) in dogs. Other reports have described undesirable side reactions when amino acids or protein hydrolysates were infused rapidly in humans. Although glutamic acid was not specifically mentioned, it is highly probable that in many cases it was largely responsible for the emesis.

With the knowledge that glutamic acid is a predominant offender, an attempt has been made to find drugs which will delay or inhibit emesis resulting from the infusion of a pure solution of the natural (*l*+) glutamic acid. Achieving this might materially enhance the clinical application of protein hydrolysates.

Methods. Healthy mongrel dogs maintained on a standard chow diet were used. Food was not withheld until 2 to 3 hours before the experiment. A 5% solution of *l*+ glutamic acid (not neutralized) having a pH of 1.1 was infused intravenously at a rate of 10 mg/kg/min until vomiting occurred. The substance to be tested as an inhibitor of emesis was either injected shortly before and/or during the glutamic acid infusion, or mixed with the solution. In order to establish individual tolerance, each dog was subjected to an infusion with glutamic acid alone, several days before or after the combined use of glutamic acid and the compound investigated. Any animal failing to vomit with glutamic acid alone was eliminated from the series.

Experimental Observations. Data recorded in Table I for *first-run* dogs show that animals given glutamic acid alone vomited when an average of 136 mg/kg had been administered at the relatively rapid rate of 10 mg/kg/min. Animals tested again within a 4- to 6-day period were found to have developed an increased tolerance (Table II). Therefore, in statistical comparison, it was necessary to use results on "first-runs" only. A rest period of 2 weeks to a month was

1 Madden, S. C., Woods, R. R., Shull, F. W., Remington, J. H., and Whipple, G. H., *J. Exp. Med.*, 1945, **81**, 439.

2 Unna, K., and Howe, E. E., *Fed. Proc.*, 1945, **4**, 138.

3 Madden, S. C., Bassett, S. H., Remington, J. H., Martin, F. J. C., Wood, R. R., and Shull, F. W., *Surg., Gynec., and Obst.*, 1946, **82**, 131.

TABLE I.
Effect of Drugs on Vomiting Threshold of Intravenous Glutamic Acid.

		Drug	Dose	No. of dogs	Mean emetic dose mg/kg	Std. error	"P" value rel. to I
I	5% glutamic acid—10 mg/kg/min	None		20	136	8.26	
II	Same	Pento-barbital Na	2 mg/kg	17	241	20.8	<0.01
III	Same, with —	Epine-phrine	10 μg/cc infus. sol'n	10	198	18.3	<0.01

TABLE II.
Vomiting Threshold in Successive Tests, Showing Rising Tolerances.

Dog	First test mg/kg	Subsequent test mg/kg	Time between tests days
1	153	180	6
3	173	286	6
4	113	132	6
5	170	135	6
6	123	127	6
7	87	113	4
14	126	164	6
16	58	124	6
20	145	166	6
21	116	128	6

found to eliminate any acquired tolerance, so that tests on the same dog after such an interval were regarded as first-run results also.

Pretreatment. (a) *Atropine.* Three dogs (6 to 9 kg) were tested with this drug. The first received 3 mg of atropine sulfate intravenously 12 minutes before infusion of glutamic acid, and 2 mg after 8 minutes of infusion. The second was given 3 mg atropine intravenously 13 minutes before infusion, and the third received 3 mg atropine intraperitoneally 8 minutes before infusion. None of them showed a significant increase in tolerance to the emetic effect of glutamic acid.

(b) *D-desoxyephedrine.* Eight dogs each received *d*-desoxyephedrine, 1 mg/kg intravenously, 10 minutes before glutamic-acid infusion. No significant increase in tolerance occurred.

(c) *3,5,5-Trimethyloxazolidine-2,3-dione, (Tridione).* This drug was tested on 2 dogs. One received 25 mg/kg intravenously 2 minutes before the infusion of the glutamic acid. The second was given 50 mg/kg 2 minutes before the infusion, and another 50 mg/kg was injected into the tubing after 14 minutes of infusion. Neither showed a significant increase in tolerance.

(d) *Pyridoxine with thiamine.* One dog was given 50 mg pyridoxine and 100 mg thiamine intravenously immediately before the glutamic-acid infusion. The second received a similar dose and in addition 3 doses of 25 mg pyridoxine plus 50 mg thiamine were injected during the infusion period. The third was treated similarly but only 2 additional injections were made during the infusion. The fourth dog received 50 mg pyridoxine and 100 mg thiamine intravenously 15 minutes before the infusion. None of the 4 dogs reacted with a significant increase in tolerance.

(e) *Pentobarbital sodium (Nembutal).* Twenty "first-run" experiments comprised this series with a dose of 2 mg/kg nembutal administered intravenously 10 minutes before glutamic acid infusion started. Table I indicates the results. All animals, without exception, tolerated an increased amount of glutamic acid before vomiting occurred. The average amount to produce vomiting was 241 mg/kg, an increase of 84% over the control level. The nembutal injection produced no detectable sedation in the dog. Four experiments were carried out with 4 mg/kg nembutal. This amount also had no sedative effect, but the increase in tolerance was not appreciably greater than with the smaller dose. In one dog, where the infusion of glutamic acid was delayed 30 minutes after the injection of

nembutal, the protective action had virtually disappeared.

(f) *Epinephrine.* During the course of the experiments both with and without nembutal premedication, it was noted that certain animals could not be induced to vomit or required far greater than average amounts of glutamic acid before vomiting occurred. In most instances this was observed in dogs which were nervous, whining or struggling. It occurred to us that the augmented release of epinephrine in these animals might be a factor in the increase in tolerance. It was decided to carry out a series of tests in which the glutamic-acid solution would contain an amount of epinephrine which normally does not produce a significant rise in blood pressure in the unanesthetized dog. The quantity used was 2 μg of epinephrine per kg per minute. It was mixed in the infusion fluid to provide 10 μg epinephrine per cc of 5% glutamic acid, which resulted in a simultaneous infusion of each substance at the desired rate. Table I shows that the addition of such an amount of epinephrine to the infusion fluid produced an increase in tolerance to glutamic acid of nearly 46% (10 dogs), the average amount of glutamic acid required to produce vomiting being 198 mg/kg. Throughout the experimental series, occasional testing of blood and urine failed to show any evidence of hemolysis.

To determine the possible effects on blood pressure and pulse rate, direct recordings from the femoral artery were made on 4 unanesthetized dogs with a Hamilton membrane manometer. Pulse rates varied less than 10% under any of the experimental conditions. Blood pressure likewise was only slightly affected. This was true of the infusion of glutamic acid either with or without epinephrine. Variations of less than 20 mm Hg on either side of the control readings were obtained, and in the same dog successive tests showed opposite responses. Respiration was not affected. In none of the experiments was the infusion continued to the point of vomiting, in order that the complicating factors incident to this reflex would be avoided. No significant hyperpnea was noticed.

Results on dogs anesthetized with phenobarbital were markedly different with respect to circulatory effects. Glutamic acid alone infused at the usual rate of 10 mg/kg/min produced a marked fall in blood pressure, which was maintained as long as the infusion continued, and the recovery was gradual. Epinephrine with the glutamic acid produced a rise of 40 to 80 mm Hg which diminished slightly as infusion progressed, but never returned to normal until the infusion was terminated. Moderate hyperpnea resulted with epinephrine, while none occurred with glutamic acid alone.

Discussion. The average dose required to produce vomiting (136 mg/kg) with glutamic acid alone, differs from values obtained by others, who used a slower rate of infusion with a neutralized solution,[2] or a mixture of other amino acids along with the glutamic acid.[1] In the present experiments it might be expected that a faster rate would not allow as much time for escape of the glutamic acid from the circulation by any route, and thus the blood concentration would be expected to rise more rapidly. It might be pointed out that the development of a temporarily increased tolerance was strictly avoided and only "first-run" data were used in the statistical analysis of results. The number of animals used in the comparison of first and second runs was not sufficient to warrant statistical analysis, but only one of 10 dogs showed a decreased tolerance on the second run, while an appreciable rise in the threshold to vomiting occurred in all the others (Table II).

The choice of the various substances tested was based in part on an anticipation of their possible use in clinical practice, where little time is available for premedication before the infusion of the nutritive materials is started. The use of pyridoxine and thiamine for several days previously might give entirely different results, but such an experiment would not represent the usual clinical possibilities. Failure of atropine to produce an improvement indicates that the emetic response to glutamic acid is not exclusively parasympathetic. Tridione, as an anticonvulsant with little sedative action, was used to investigate possible cortical involvement. *D*-desoxyephedrine

as a central nervous system stimulant provided another avenue of approach.

The mechanism by which nembutal increases the tolerance to glutamic acid cannot be established with certainty from the present experiments. The drug may exert a depressant action directly upon the vomiting center or elsewhere along the nervous pathways, thus delaying the onset of the reflex by elevating the threshold. Another possible action would be a depression of the motility of the gastro-intestinal tract, which has been observed both *in vivo* and *in vitro* by Gruber and Gruber.[4,5] Shaw[6] has shown the site of depressant action to be both in the postganglionic fibers and in the muscle cells themselves.

The effect of epinephrine may be due to sympathetic predominance causing suppression of the vomiting reflex, with the action either central or peripheral, or both. The simultaneous administration of epinephrine, as outlined above, possesses the theoretical advantage that its action is limited to the actual duration of the infusion, because of its rapid inactivation in the body, whereas other agents given beforehand may either exert their effect still for some time after the need for them is ended, or may have such a brief effective period that a careful timing is necessary to take full advantage of the protective action. However, the physiological considerations involved in the administration of epinephrine might impose restrictive limitations upon its clinical usefulness.

From the results of 111 experiments obtained with these various agents, it would appear that the emetic effect of glutamic acid may be prevented or ameliorated in several possible ways. The administration of a suitable sedative, prior to the infusion of protein hydrolysates or amino acid mixtures prone to produce vomiting, might be attempted clinically in order to improve tolerance. This could be done either by injection or by rectal suppository.

Summary. 1. *l*+ glumatic acid, when infused at a rate of 10 mg/kg per minute in dogs induced vomiting on an average when 136 mg/kg had been administered.

2. Atropine, *d*-desoxyephedrine, tridione, or pyridoxine with thiamine had no significant effect in delaying vomiting.

3. When nembutal, 2 mg/kg was given intravenously 10 minutes before the glutamic-acid infusion, vomiting was not induced until an average of 241 mg/kg had been given, an 84% improvement in tolerance.

4. Epinephrine, infused with glutamic acid in a concentration of 2 μg/kg/min delayed vomiting until an average of 198 mg/kg had been infused, a 45% tolerance increase.

5. Tolerance to glutamic acid increased if infusions were repeated within 4 to 6 days.

6. The possible applications of these findings to the clinical use of protein hydrolysates are briefly discussed.

[4] Gruber, C. M., Jr., and Gruber, C. M., *Arch. Int. Pharmacodyn.*, 1939, **63**, Fasc. 2, 243.

[5] Gruber, C. M., and Gruber, C. M., Jr., *J. Pharm. and Exp. Therap.*, 1941, **72**, 176.

[6] Shaw, F. H., *Austral. J. Exp. Biol. and Med.*, 1942, **20**, 117.

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Experimental Acceleration of Secretion of Urine in Fetal Rats.*

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In the fetal rat, ligation of the ureter causes the development of hydroureter and hydronephrosis, while ligation of the urogenital papilla causes the storing of urine† in the

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† In collaboration with Dr. Gerald T. Evans and Miss Harriet Lamberg, the chemical composition