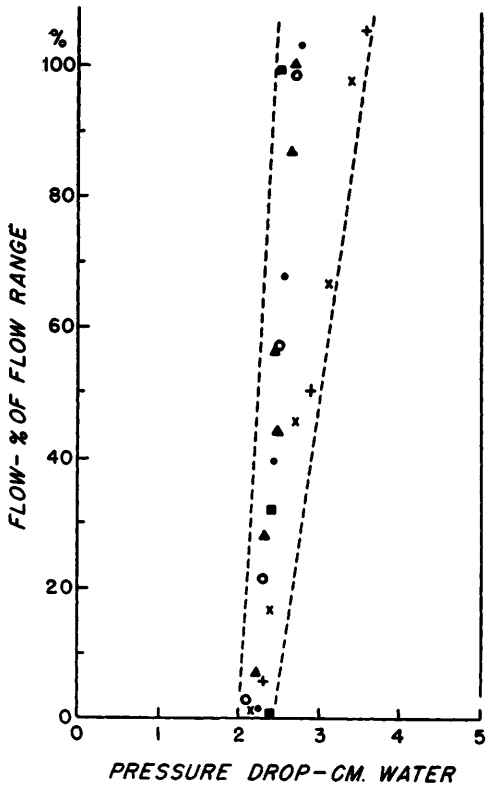

FIG. 5. Pressure drop through rotameter with different metering chambers and at different rates of flow. Ordinate: flow is expressed in terms of percentage of the nominal capacity of each metering chamber. Dots: metering chamber with nominal capacity of 100 cc/min; open circles: 200 cc/min; squares: 400 cc/min; triangles: 800 cc/min; plus signs: 1600 cc/min; ×'s: 3 liters/min.

that the connecting tubing and cannulas used in association with the rotameter be of maximum bore and minimum length.

In different acute experiments and under a variety of conditions, satisfactory continuous records have been made of the rate of blood flow through carotid, renal, femoral, and coronary arteries and through jugular, venacaval, renal, femoral, and coronary sinus veins. Illustrations of typical records may be found in other reports(3-6).

Summary. (1) A simplified and improved flow rate meter is described. The instrument is basically a rotameter, the float of which is vertically displaced in proportion to the rate of flow and its position detected electromagnetically. The rate of flow is indicated on a microammeter and a recording galvanometer. The metering chamber and float are designed so that viscosity effects are minimized and the pressure drop through the instrument reduced to less than 3.5 cm of water at any rate of flow. (2) To obtain maximum sensitivity over a given flow range, different metering chambers, with capacities ranging from 100 cc to 3 liters per minute, can be used interchangeably with one detecting unit.

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Prevention of DL-Methionine Toxicity in Rats by Vitamins E, B_{12}, Folacin, Glycine and Arginine.* (19197)

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The toxic effect of high levels of DL-methionine was noted by Earle, Small, and Victor

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(1). Brown and Allison(2) showed that 1.7% dietary arginine partially prevented loss in weight of rats on a diet containing 12% casein and 4.8% DL-methionine. They also noted an increase in creatinine excretion of rats on the high methionine diet. Van Pilsum and Berg(3) and Cohn and Berg(4) recently reported that the toxic effects of excess dietary (1.8%) DL-methionine in rats were counteracted by the addition of molecular equivalent quantities of glycine and arginine to the diet, while guanidoacetic acid was ineffective. Their diet contained ample quantities of vit. E, folacin, and vit. B_{12}.

It has been shown(5) that methionine protects against the acute toxicity of carbon tetrachloride under special dietary conditions. Vit. E and vit. B_{12} share this property(6). Hove(7) noted the similarity of the symptoms of vit. E deficiency and carbon tetrachloride poisoning in rats. Data at this laboratory (8) show that vit. E increases the utilization of methionine in methylating guanidoacetic acid to creatine *in vitro*. Hove and Hardin (6) found that vit. E and folacin stimulated the growth of young rats on a 10% protein diet; therefore, it became of interest to determine if vit. E, folacin, and vit. B_{12} influenced the toxicity of excess methionine.

Experimental. The basal diet contained 10% casein purified as reported by Salmon (9), 9% lard, 1% cod liver oil, 4% salt mixture(9), 0.5% DL-methionine, and sucrose to 100%. The basal diet contained the following levels of pure vitamins per g: thiamine, riboflavin, and pyridoxine, 5 μg each; calcium pantothenate, 25 μg; niacin, 40 μg; choline chloride, 2 mg; i-inositol, 0.2 mg; and 2-methyl 1-4 naphthoquinone, 2 μg. The supplements used were added to the basal diet as follows: α-tocopherol acetate, 0.01%; crystalline vit. B_{12}, 3 μg %; and folacin, 0.1 mg %. In addition to the methionine furnished by the casein (about 0.3%), the diet was supplemented with 2.5% DL-methionine or 2% above the level in the basal diet. Glycine (1.0%) and L (+) arginine (2.3%) were added in some cases. These were molecularly equivalent to 2% methionine. Weanling albino rats of Sprague-Dawley origin weighing 45 $\pm$ 5 g were fed the basal diet until average weight was 65 $\pm$ 5 g. At that time the rats were distributed among the dietary treatments according to sex and litters. A total of four experiments were conducted with 3 to 4 rats per dietary treatment. The rats were housed individually in metal cages and food and water were provided *ad libitum*. The animals were weighed at the beginning of the experiment and weekly thereafter. At the end of 4 weeks, urinary creatine analyses were made by the method of Eggleton, Elsden and Gough (10); 2 ml of carbon tetrachloride per kg body weight were then injected and urinary creatine was again determined on the rats surviving 24 hours. The number of deaths that occurred during the first 48 hours following injection of carbon tetrachloride were recorded.

Results. When the DL-methionine was increased by 2 percentage points in the basal diet, growth rate of the rats for the 4-week period was decreased from 61 g to 22 g, or a decrease of 64% (Table I). When α-tocopherol acetate, folacin, and vit. B_{12} were added to the high methionine diet, the growth inhibition due to toxicity of the methionine was 39%. A statistical analysis of the data by means of Fisher's t test indicated that this was a highly significant protective effect. A t value of 4.7 was obtained on comparing the high methionine groups with and without the combined vitamin supplements.

When the vitamins were added to the high methionine diet either individually or in pairs, it was noted that the folacin-supplemented diet gave slight, but significant protection against methionine toxicity (t value 2.9). However, when α-tocopherol acetate was combined with folacin, very significant partial protection was obtained (t value 9.0). α-Tocopherol acetate and vit. B_{12}, alone or in combination, were without effect on growth.

The addition of molecular equivalent quantities of glycine and arginine gave a very significant partial protection (t value 7.0) against the high methionine, which was approximately the same as that obtained by the vitamin supplements. When supplements of tocopherol acetate, folacin, and vit. B_{12} were

TABLE I. Influence of Dietary Supplements on Toxicity of DL-Methionine, Creatine Excretion and Sensitivity to Carbon Tetrachloride in Rats.

Addition to 10% casein diet	No. rats	4 wk gain g	4 wk gain S.E. ±	Urine creatine (mg/day/100 g body wt) Without CCl_4	Urine creatine (mg/day/100 g body wt) With CCl_4*	Survivals 48 hr after 2 ml CCl_4/kg body wt No.	Survivals 48 hr after 2 ml CCl_4/kg body wt %
DL-meth. (.5%)	14	61	4.7	1.5	5.4	6	43
" + vit. E, B_{12}, folacin	14	69	2.8	.7	2.3	14	100
DL-meth. (2.5%)	15	22	1.5	.8	11.0	3	20
" + vit. E, B_{12}, folacin	15	37	2.8	.5	4.3	8	53
" + vit. E	11	22	.7	.3	.3	6	55
" + vit. B_{12}	11	22	1.3	.2	0	5	45
" + folacin	11	27	.9	.1	.4	4	36
" + vit. B_{12} and folacin	11	25	.6	.2	.4	5	45
" + vit. E and B_{12}	11	23	1.3	.2	—	2	18
" + vit. E and folacin	7	38	1.3	.2	—	1	14
" + gly. and arg.	4	43	2.6	2.7	17.2	1	25
" + gly. and arg. + vit. E, B_{12}, folacin	4	66	5.9	4.6	3.8	4	100
" + guanidoacetic acid	4	16	5.6	31.0	28.9	1	25
" + guanidoacetic acid + vit. E, B_{12}, and folacin	4	32	3.2	28.1	15.3	3	75

* Urine collected for 24 hr after the CCl_4 injection.

supplied in combination with the glycine and arginine, the growth inhibition due to the excess methionine was eliminated, which confirms the work of Cohn and Berg(4). Guanidoacetic acid did not protect against methionine toxicity. The addition of the 3 vitamins along with the guanidoacetic acid produced significantly higher growth than the guanidoacetic acid alone, (t value 6.3).

The urinary creatine of the rats receiving high methionine was decreased to about half the value of the urinary creatine of rats on the basal diet. The urinary creatine of the rats receiving glycine and arginine was increased above the level in the control rats. The addition of α-tocopherol acetate, folacin, and vit. B_{12} increased the urinary creatine even more. The addition of guanidoacetic acid to the basal diet produced a very high excretion of apparent creatine. Of this apparent creatine, about half could have been due to the excretion of guanidoacetic acid, since the method used for creatine analysis will give a color with guanidoacetic acid equivalent to 1/10 that produced with creatine. The high methionine diet afforded less protection against creatinuria caused by carbon tetrachloride than did the basal diet. In all cases, the vitamins reduced the creatinuria due to CCl_4.

The percentage of rats surviving in each group at the end of 48 hours after injections of 2 ml carbon tetrachloride per kg body weight is also shown in Table I. The addition of the 3 vitamins to the basal diet afforded 100% protection against this level of carbon tetrachloride. Rats on the high methionine diet were more sensitive to carbon tetrachloride than were rats on the basal diet except when the diet was supplemented with glycine, arginine, and the combined vitamins. Vit. E, folacin, or vit. B_{12} seem to have comparable protective effects against the toxicity of carbon tetrachloride given to the rats on the high methionine diet. Combinations of vit. E with vit. B_{12} or folacin were without effect.

Since glycine was involved in protecting rats against methionine toxicity, the reverse was studied. The data in Table II show that either α-tocopherol acetate (.01%) or DL-methionine (1.0%) prevented death due to toxicity of 4% dietary glycine. Partial protection against the growth depression due to this level of glycine was provided by either supplement, while the combination of vit. E and methionine gave complete protection.

Discussion. There may be several explanations of why glycine and arginine, in the presence of the 3 vitamins, protect against excess DL-methionine.

TABLE II. Glycine Toxicity in Rats Counteracted by Vitamin E and Methionine. 4 to 6 rats per group.

Additions to 10% casein diet	4 wk weight gain with % dietary glycine at 0	4	Depression of growth rate due to glycine (%)
None	36 g	24 g*	33
Vit. E, .01%	45	34	24
DL-methionine, 1%	64	49	23
Vit. E and methionine	69	63	8

* Five of the 6 rats in this group died during the 3rd and 4th wk. The value given is the 3-wk gain extrapolated to 4 wk.

An hypothesis advanced by Cohn and Berg (4) is that the detoxification of excess methionine is accomplished by supplying the proper substances to utilize the methyl groups. Data from this laboratory(5) show that α-tocopherol acetate increases the utilization of methionine in the synthesis of creatine *in vitro* from guanidoacetic acid. The growth data show that supplementary vit. B_{12} or vit. E, alone or together, are not effective in detoxifying methionine. It can be reasoned that, since the precursors of creatine, *i.e.*, glycine and arginine, in the presence of the combined vitamins are very effective in detoxifying methionine, the detoxification is accomplished by using up excess methyl groups to form creatine. The urinary creatine was increased when glycine and arginine were fed. It was increased still more when vit. E, folacin, and vit. B_{12} were added along with the glycine and arginine. This increase was approximately proportional to the increase in body weight caused by the addition of these factors.

An alternate explanation could be that the high methionine creates an amino acid imbalance and precipitates glycine and arginine deficiencies in the animal.

Guanidoacetic acid may not take the place of the glycine and arginine in detoxifying the methionine because these specific amino acids have become dietary essentials. It could be that, for the synthesis of creatine, the reaction of the guanidyl group from arginine with the acetate and methyl groups from glycine and methionine, respectively, is a coupled reaction and will take place more readily when all of these are present simultaneously.

Summary. (1) The toxicity of DL-methionine, fed to rats at a 2% dietary excess level, was more pronounced in the absence of dietary vit. E, folacin, and vit. B_{12}. The combination of vit. E and folacin was as effective for growth as all 3 vitamins together. Without the 3 vitamins, growth in 4 weeks was 32% of the normal, as compared with 54% of normal upon their addition to the diet. Supplements of glycine and arginine in the absence of these vitamins produced growth that was 62% of normal. (2) The methionine toxicity was prevented by molar equivalent supplement levels of glycine plus arginine if the 3 vitamins were added to the diet (95% of normal), but was not prevented in the absence of these factors. (3) Urinary creatine and sensitivity to acute carbon tetrachloride were determined for all rats. (4) Complete protection against glycine toxicity was given by dietary methionine with vit. E.

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