sulfate for 98 hours. On the other hand, when trimmed roots from the same lot were subjected to the same conditions, no significant change in glutamine was noted in spite of a clearly demonstrable increase in ammonia. The data for this experiment were obtained from the analysis of dried samples and doubtless represent an underestimate of the actual quantities of glutamine present.

It should perhaps be pointed out that experiments of this kind with field grown beet plants are frequently unsatisfactory owing to the inhomogeneity of the material with respect to glutamine content. When small lots are employed, the most careful selection of plants for apparent similarity may fail to give samples that duplicate each other sufficiently closely for reliable results. However, the experiment has been repeated a number of times and consistent behavior observed. Although the data given in detail in Table I are from an experiment that was by no means as striking with respect to the magnitude of the increase in glutamine as was observed in some of the other trials, they furnish the clearest evidence that has yet been presented of the course of the phenomenon and demonstrate that the effect of *in vitro* culture of beet roots with tops attached in ammonium salt solutions follows the same pattern after the plants have been removed from the soil as it does in undisturbed plants in the field. Furthermore, it is clear that treatment in this way of beet plants that are to be used for the preparation of glutamine in quantity is greatly to be desired if high yields are sought.

Special emphasis should perhaps be given to the point that determinations of glutamine amide nitrogen in beet root tissue that has been subjected to drying at 80°C are unreliable as an index of the glutamine content of the fresh tissue because of the danger of hydrolysis of a part of the glutamine during the drying process. It is probable that earlier published data obtained in this way are somewhat low.

Summary. The glutamine content of beet root tissue to be used for the preparation of glutamine on the large scale can be materially increased if the roots of the plants with tops attached as purchased in the market are immersed in 0.15 M ammonium sulfate solution for from 4 to 6 days before being trimmed and extracted with water. Tissue that will yield 5 g or more of glutamine per kg can be prepared in this way. A demonstration is afforded that, contrary to earlier observations, significant losses of glutamine are experienced if beet root tissue is dried at 80°C in preparation for analysis.

16462

Plasma Methionine After Oral Administration of *DL-Methionine* in Human Subjects.

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In a recent publication[1] plasma methionine levels after the intravenous administration of 1.5 g of *DL*-methionine were reported for normal human male subjects. In the present communication there is reported a similar study of plasma methionine levels in both male and female normal subjects after oral administration of the amino acid. A few observations on abnormal subjects are included for comparative purposes.

Limited conclusions on the absorption of methionine from the intestine may be drawn

[1] Harper, H. A., Kinsell, L. W., and Barton, H. C., *Science*, 1947, **106**, 319.

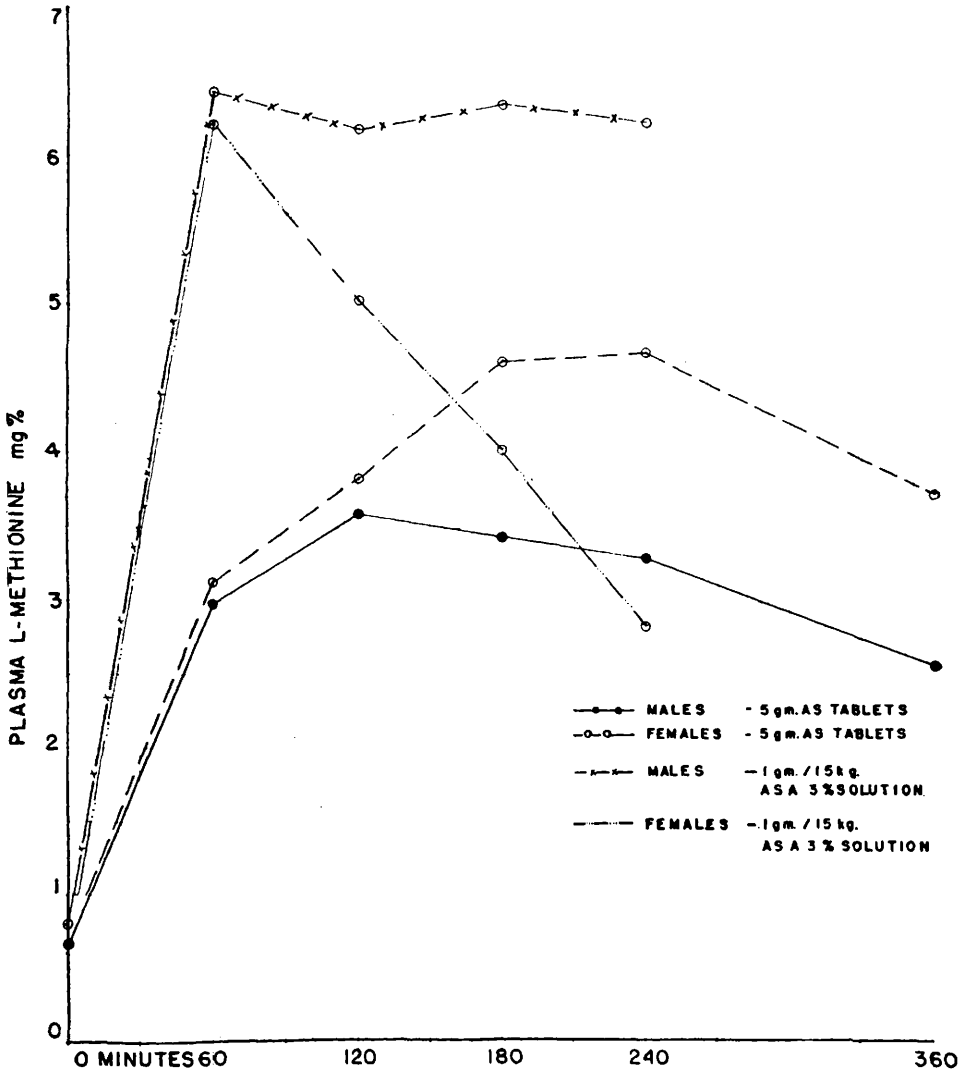

FIG. 1.

Average plasma *L*-methionine levels prior and subsequent to oral administration of *DL*-methionine to normal male and female subjects.

from this study since coincident with the rise in plasma levels by absorption there is a variable rate of uptake by the tissues as well as excretion, particularly of the *D* isomer, both tending to lower the plasma methionine. A similar study of human blood glycine levels after oral administration of this amino acid was recently reported by Gutman and Alexander.[2] Blood tyrosine levels were followed by Bernhart and Schneider[3] in connection with the development of a tyrosine tolerance test. Using the dog as the experimental animal, Hier[4] reported blood values for many individual amino acids after oral administration of a given test amino acid. It is noteworthy that the behavior of methionine was unique in comparison to the other amino acids studied.

Method. Normal subjects for study were

[2] Gutman, G. E., and Alexander, B., *J. Biol. Chem.*, 1947, **168**, 527.

[3] Bernhart, F. W., and Schneider, R. W., *Am. J. Med. Sci.*, 1943, **205**, 636.

[4] Hier, S. W., *J. Biol. Chem.*, 1947, **171**, 813.

TABLE I.
Plasma *L*, and Apparent *D*, (*DL-L*) Methionine Levels Prior and Subsequent to Oral Administration of 5 g *DL*-Methionine Tablets.

		Time (min.)										
		0	60		120		180		240		360	
Subject	Sex	*L*	*L*	*D*	*L*	*D*	*L*	*D*	*L*	*D*	*L*	*D*	Remarks
		mg/100 cc										
B.	M	0.56	2.46		3.00		2.84		3.12				Normal
L.	M	1.32	3.04	2.24	3.22	2.98			3.02	3.28	2.10	2.40	”
U.	M	0.50	3.52		4.40				3.88		3.28		”
K.	M	0.28	2.88		3.76				3.20		2.32		”
A.	F	0.56			5.40	5.60	6.30	5.10	5.50	4.70	4.80	5.00	”
Lo.	F	1.04	3.76	2.54	3.36	2.34	4.48	3.42	5.84	0.96	3.76	1.24	”
Le.	F	0.42	3.28		3.88	2.32	4.24	2.46	4.16	2.64	3.40	1.30	”
Q.	F	0.92	3.32	1.88	3.26	2.54	3.30	4.50	2.60	11.60	2.10	17.90	”
Hn.	F	0.30	2.20		3.24		4.88		5.44		4.68		”
C.	M	1.88	4.64	3.86	5.60	3.40	6.48	5.12	6.36	3.24	4.68	4.92	Cirrhosis
Mk.	F	0.20	2.48		3.88	3.22			4.52	2.28	5.24	3.46	Ulcerative colitis
Me.	F	0.32	1.76				3.50		4.88				Massive intestinal resection

7 male and 8 female patients without any demonstrable abnormality which might be expected to influence the results of the test. All were fasted 12 hours before the test dose of methionine was administered and no food was taken during the subsequent period of study. In the first series of experiments a standard dose of 5 g of *DL*-methionine tablets was given, the subject merely swallowing 10, one-half gram tablets followed by a glass of water. In the second series a 3% solution of *DL*-methionine was administered on the basis of body weight in the amount of 1 g per 15 kg. Blood was drawn for a determination of the fasting methionine level and, subsequent to the test dose, at intervals of 60, 120, 180 and 240 minutes with additional samples at 30, 90 and 360 minutes in some cases. Microbiological determinations of plasma *L*-methionine were made employing methods previously described.[1] *Leuconostoc mesenteroides* P-60 was used as test organism for the assay of *L*-methionine and for the *DL* compound, *Lactobacillus fermenti* 36.[5] The difference between the *DL* and *L* values permits estimation of the apparent concentration of the *D* isomer.

Results and Discussion. Fig. 1 depicts the average plasma *L*-methionine levels in normal male and female subjects prior and subsequent to the ingestion of 5 g *DL*-methionine tablets as well as after 1 g/15 kg body weight, *DL*-methionine in a 3% solution. Since relatively few determinations were made at 30 or 90 minutes after the ingestion of the test dose of methionine, these values were eliminated from the curves illustrated in Fig. 1 as well as from the tables to follow. However, in all but one case (Q. female, Table II) plasma values at 60 minutes exceeded those observed at 30 minutes suggesting that absorption was still proceeding at the earlier period. Although the dosages were higher when the tablets were given, the plasma methionine concentration was observed to decline before reaching levels equivalent to those after the solution was administered. In addition in this latter case, there was a tendency for the peak levels to be reached sooner. These observations suggest more prompt and possibly more efficient absorption of the amino acid solution.

The curves illustrating the variation in plasma *L*-methionine after oral administration were somewhat different when males and females were compared. A more rapid rate of disappearance of the administered methionine was observed in females, particularly marked after the solution had been ingested. On the tablet dose, there was a tendency towards the

[5] Dunn, M. S., Camien, M. N., Shankman, S., and Block, H., *J. Biol. Chem.*, 1946, **163**, 577.

TABLE II.
Plasma *L*, and Apparent *D*, (*DL-L*) Methionine Levels Prior and Subsequent to Oral Administration of a 3% Solution of *DL*-Methionine (1 cc/15 kg actual weight).

Subject	Sex	Dose, g	Time (min.) 0 *L*	60 *L*	60 *D*	120 *L*	120 *D*	180 *L*	180 *D*	240 *L*	240 *D*	Remarks
			mg/100 cc									
Hl.	M	5.3	0.50	6.40	12.90	6.10	8.80	6.00	7.50	6.08	4.22	Normal
Z.	M	4.2	0.94	6.88	7.92	6.32	7.18	7.44	2.16	6.48	3.62	"
U.	M	5.0	0.40	6.00	5.80	6.24	4.56	5.76	4.44			"
Lo.	F	3.7	1.12	5.28	9.22	5.76	4.34	4.24	3.76	2.24	4.24	"
W.	F	3.3	0.92	6.08	6.72	4.72	5.38	4.64	2.06	3.68	0.32	"
Q.	F	3.6	0.40	7.36	5.34	4.72	3.78	3.28	4.02	2.64	4.56	"
K.	F	2.7	0.34	8.64	3.36	5.68	3.12	5.28	2.32	3.60	3.36	Ulcerative colitis
Ha.	F	3.7	1.32	10.40	7.00	9.44	3.96	4.16	7.04	9.28	5.92	Carcinoma of gall bladder
D.	F	2.9	0.48	2.88	2.80	2.72	4.08	1.84	4.44	1.44	4.44	Carcinoma of pancreas
S.	M	3.2	0.44	3.72	4.88	3.84	3.96	3.42	4.18	2.96	1.74	Fever of undetermined origin

attainment of higher plasma levels and at rates slower than those noted in the male on the same dosage. This may be due to the fact that the 5 g dose was high in proportion to size and hence that absorption was more prolonged.

In Hier's experiments[4] very large doses (0.56 g per kg) of methionine were given to the dog and it was noted that, in contrast to any other amino acid studied, blood levels of methionine remained considerably elevated for as long as 24 hours. The doses used in the experiments here reported were proportionately much lower since it was desired to duplicate conditions which would be expected in therapeutic practice. In this dosage range in normal individuals no particular intolerance to methionine has been observed.

Tables I and II detail the plasma *L* and apparent *D* (*DL-L*) methionine levels obtained after the 5 g oral dose or the 1 g/15 kg dose administered orally as a 3% solution. From these data there is no evidence to suggest any variation in rate of absorption of the two isomers. The plasma levels of the apparent *D*-methionine are much more variable than those observed for the *L* isomer. This is certainly due in part to the fact that the rate of removal of the apparent *D* isomer is much more dependent on excretion by the kidney than is the *L* isomer wherein this factor can be neglected at the dosages employed.[6] It is therefore difficult to assign significance to the apparent *D*-methionine plasma levels since, although the rate of absorption seems equal to that of the *L* isomer, the rate of removal from the plasma is influenced not only by metabolic phenomena attendant upon its utilization but also by excretion. Variation in plasma *L*-methionine seems therefore a better indication of the normal physiological phenomena attendant upon absorption and utilization.

Observations on a few abnormal subjects are also presented using both experimental technics. Patient *Me*, female, Table I, had sustained a massive resection of the small intestine, only about 18 inches of the small bowel remaining. However, as evidenced by the increasing plasma levels, absorption was maintained up to the fourth hour suggesting absorption from the colon. That amino acids are absorbed to some extent from the colon was shown by Short and Bywaters in studies of rectal feeding.[7] The male subject with a diagnosis of cirrhosis exhibited a high fasting methionine level and somewhat elevated concentrations after ingestion of the test dose with an initial slow rate of disappearance. This is in accord with other observations in

[6] Kinsell, L. W., Harper, H. A., Barton, H. C., Michaels, G. D., and Weiss, H. A., *Science*, 1947, **106**, 589.

[7] Short, A. R., and Bywaters, H. W., *Brit. Med. J.*, 1913, **1**, 1361.

hepatic disease using intravenously administered methionine.[6] The female patient with ulcerative colitis presented a fairly normal curve except for the delayed peak reached at 6 hours.

In studies of abnormal subjects using the solution dose (Table II), certain definite deviations from normal were noted. In a case of carcinoma of the gall bladder, extremely high plasma levels of methionine were reached with a rapid fall between 120 and 180 minutes followed by a secondary rise at 240 minutes. In marked contrast, the patient with carcinoma of the pancreas exhibited extremely low plasma levels of methionine with a steady rate of disappearance. A case of ulcerative colitis showed a high initial peak but no other noteworthy abnormality in plasma levels. The last subject with fever of undetermined origin showed a somewhat flat curve suggesting some decrease in intestinal absorption although increased utilization due to an accelerated metabolic rate cannot be excluded.

The abnormal cases presented are not numerous and are intended to serve mainly as comparisons to the normal controls. Further study of this phase of the problem is necessary to establish the validity and significance of the apparent deviations from the normal pattern which have been pointed out.

Summary. 1. The concentration of methionine in the plasma after oral administration of *DL*-methionine has been studied in humans.

2. The results suggest more rapid and more efficient absorption of the amino acid when administered in solution rather than in tablet form.

3. A faster rate of disappearance of the administered methionine from the plasma was noted in the female subjects.

4. Observations on the apparent *D*-methionine levels suggest that the *D* isomer is absorbed at a rate equal to that of the *L* form. However, there is considerable individual variation in the rate of disappearance of the *D* isomer from the plasma.

16463 P

Sodium Oxalate as Anticoagulant.

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It is well known that 0.0025 M solution of sodium oxalate does not delay blood coagulation, even when in stoichiometric concentration with calcium. Three times more sodium oxalate than calcium present in blood is required to prevent blood coagulation (Quick[1]). On the other hand the prothrombin time of oxalated human plasma is prolonged because destruction of the plasmatic cofactor of thromboplastin (P.C.T.) starts a few hours after storage.[3] Quick believes that calcium protects this factor because hemophilic plasma from which calcium has not been removed does not alter its prothrombin time after 48 hours.[2] These experiments do not explain satisfactorily why an excess of sodium oxalate is needed to render blood incoagulable and why oxalated stored plasma destroys by oxidation its cofactor of thromboplastin.[3]

We do not believe that the prothrombin time of oxalated human stored plasma is prolonged because calcium is eliminated. Another possibility must be considered. Sodium oxalate may have more affinity for another substrate than calcium and only when an excess has been added does this last reaction take place. It seems logical to suppose the existence of a

[1] Quick, A. J., *The Hemorrhagic Diseases and the Physiology of Hemostasis*, 1942, Baltimore, Thomas, Ed.

[2] Quick, A. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 249.

[3] Honorato, C. R., *Am. J. Physiol.*, 1947, **150**, 381.