TABLE I.
Titration of the Hamster-Adapted Newcastle Virus of the 200th Passage.

Hamsters Age in weeks	Dilutions 10-1	10-2	10-3	10-4	10-5	10-6	Titer[3]
4	5/5	3/3	4/5	2/2	0/6	0/6	$10^{-4.25}$
12	5/5	4/4	2/2	0/6	0/5	0/5	$10^{-3.5}$

Numerator—Number of deaths. Denominator—Total number inoculated.

Summary. A California strain of Newcastle virus (No. 11,914) was carried serially by intracerebral inoculation through 200 passages in Syrian hamsters. The virus produced symptoms of irritability followed by involuntary motor reactions and paralysis. During the state of paralysis and while moribund, a large percentage of the infected hamsters showed marked labored breathing. The incubation period decreased gradually from 2 to 6 days in the early passages to approximately 12 hours after the 190th passage. Despite the apparent increased infectivity with progressive passages, the hamster-adapted Newcastle virus of the 200th passage titered only slightly higher in 4-week-old hamsters than that of the 49th or 72nd passages.

After the 92nd passage the hamster-adapted Newcastle disease virus was non-pathogenic for chickens when inoculated intramuscularly or subcutaneously.

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Artificial Enrichment of Beet Root Tissue with Glutamine.

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Current interest in glutamine as a metabolite in animal tissues has led to a demand for this substance for experimental purposes. Glutamine may be prepared in quantity from beet root tissue by a method modified from the original procedure of Schulze and Bosshard[1] by Vickery, Pucher and Clark[2] but the yield obtained is dependent upon the richness of the material employed and this may vary from about 2 g per kg of trimmed root tissue to as much as 5 g. However, samples that contain more than 3 g per kg are not common.

It was observed[3] some years ago that the roots become notably enriched in glutamine if beet plants are treated in the field with 2 M ammonium sulfate solution at the rate of approximately a ton of nitrogen per acre (37 liters of solution per 100 square feet), the plants being collected 4 to 10 days later. Under these circumstances, the glutamine content increased from less than 2 to about 3 g per kg. Beet plants growing in the greenhouse and treated daily for 18 days with 0.5 M ammonium sulfate in such a way that the cumulative supply of nitrogen was somewhat greater than was used in the field experiment yielded roots that contained as much as 8 g per kg of glutamine or the equivalent of 5.4% of the dry weight. However, the opportunity to prepare beet plants in this manner for glutamine isolation is seldom available to workers in biochemical laboratories, and it

* Died November 20, 1947.

1 Schulze, E., and Bosshard, E., *Ber. chem. Ges.*, 1883, **16**, 312.

2 Vickery, H. B., Pucher, G. W., and Clark, H. E., *J. Biol. Chem.*, 1935, **109**, 39.

3 Vickery, H. B., Pucher, G. W., and Clark, H. E., *Plant Physiol.*, 1936, **11**, 413.

TABLE I.
Enrichment of Beet Root Tissue in Glutamine by Culture of Plants for 138 Hours in 0.15 M Ammonium Sulfate.

Method of preparation for analysis	Before culture		After culture	
	Dried	Extracted	Dried	Extracted
Fresh wt root tissue, g	350	420	371	399
Dry wt, g	62.4		58.7	
Ammonia N, g/1000 g fresh wt	0.150	0.018	0.797	0.787
Glutamine, g/1000 g fresh wt	3.15	4.10	4.20	5.24
Asparagine, g/1000 g fresh wt	0.47	0.87	0.85	0.47
Loss of glutamine on drying tissue, g	0.95		1.04	
Loss of glutamine on drying tissue, %	23.2		19.8	
Increase of glutamine on culture, g/1000 g fresh wt			1.05	1.14
Increase of glutamine on culture, %			33	28

therefore seemed desirable to devise a method of treatment that could be applied to the plants as purchased at the market, and at any time of the year that they are available.

After preliminary experimentation on the concentration of ammonium sulfate solution and the time necessary to produce worthwhile effects, 24 beet plants selected for uniformity in size and development from a lot obtained in the local market were sorted into 2 equal groups one of which was put into an enamel ware pail that contained 3 liters of 0.15 M ammonium sulfate solution so that the roots were immersed and the tops supported by the walls of the pail. This sample was then placed in the greenhouse where it remained for 6 days. Presumably, any sunny place would have been equally suitable.

The second sample, to be used as a control, was trimmed of leaves and small fibrous roots and the main root was cut lengthwise in half to provide 2 similar subsamples. One of the portions of half roots was then sliced thin and dried at 80°C in a ventilated oven until crisp and was ground to powder in a Wiley mill. The other portion was ground in a meat chopper, the mass was treated with sufficient ether to saturate the aqueous phase in order to plasmolyze the cells and, after 30 minutes, was enveloped in drilling and pressed between steel plates in the hydraulic press. The press cake was shredded, thoroughly moistened with water and pressed again, and this washing operation was repeated a second time. The total volume of extract and washings was 1000 ml. The extract was immediately analyzed for ammonia and for glutamine and asparagine amide nitrogen. The same analytical determinations were made on the dried control sample.

At the end of the period of culture in ammonium sulfate, the other lot of beet plants was treated in the same way save that the roots were thoroughly washed to remove culture solution before being trimmed and prepared for analysis. The data are given in Table I and support the following conclusions. The glutamine content of beet root tissue can be appreciably increased by culture of the whole plant in 0.15 M ammonium sulfate solution for approximately a week even when, as in the present case, the initial glutamine content of the tissue was unusually high. The apparent glutamine content of beet root tissue is seriously diminished if the tissue is dried at 80°C, doubtless because of the instability of glutamine in the presence of water at elevated temperatures. Nevertheless, if the samples before and after treatment are prepared for analysis in the same way, an equally valid demonstration of the enrichment is provided, whether the material is dried in the oven or extracted with water at room temperature. Ammonia is freely taken up by the roots under the conditions described and is, in part, employed for the synthesis of glutamine. However, no significant increase in asparagine was observed.

The presence of the leaf tissue is essential for the reaction. A similar experiment gave evidence for an increase from 2.4 g per kg of fresh root tissue in the control to 4.3 g in the roots of the plants with leaves attached which were treated with 0.15 M ammonium

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.

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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.