TABLE I. Amino Acid Composition of Protein of Turnip Yellow Mosaic Virus. All values are expressed on a moisture-free basis. Nitrogen content, 16 g/100 g protein.

	Amino acid			
	g/100 g protein	g residue /100 g. protein	g N/100 g protein	Moles residue/10^5 g protein
Glycine	2.90	2.20	.54	38.6
Alanine	5.70	4.54	.91	64
Valine	8.45	7.14	1.01	72.1
Leucine	10.30	8.89	1.10	78.6
Isoleucine	10.80	9.31	1.15	82.4
Phenylalanine	3.80	3.39	.32	23
Proline	10.70	9.04	1.30	93
Tryptophan	1.14	1.04	.16	5.6
Cystine	2.50	2.31	.29	10.4
Methionine	2.85	2.50	.27	19.1
Glutamic acid	9.60	8.41	.92	65.2
Aspartic acid	5.10	4.41	.54	38.3
Histidine	2.10	1.86	.57	13.5
Lysine	10.70	9.40	2.05	73.2
Arginine	2.50	2.24	.80	14.4
Serine	8.10	6.71	1.08	77.1
Threonine	15.10	12.80	1.77	126.8
Tyrosine	2.45	2.20	.19	13.5
Amide NH_3	1.25	1.25	1.03	73.5
Total	116	99.6	16	982

Free basic groups, 101; free acid groups, 30; aliphatic hydroxy groups, 204.

the role that these amino acids might play in the biological activity of the proteins in which they are found in large amounts.

Summary. 1. The amino acids of the protein of crystalline turnip yellow mosaic virus were studied by paper chromatographic and microbiological technics. 2. Virtually all the protein was accounted for by microbiological assay for 18 amino acids performed on hydrolysates of the protein. 3. The protein was characterized by the presence of large amounts of serine, threonine, and proline. Hydroxyproline was not detected.

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Preparation of a Stable Form of Inulin for Tissue Analysis.* (19537)

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Direct analysis of tissue for inulin is essential for a correct evaluation of the volume distribution of this substance. Ross and Mokotoff(1) have described a method of analysis for muscle tissue using dilute alkali and heat to destroy the high inulinoid blank of this tissue. They were unable to obtain adequate recoveries of inulin added to muscle tissue by this method, unless the inulin had been previously treated with heat and dilute NaOH and reassayed before adding it to the muscle. Therefore, the analysis of the tissue of animals injected with the usually available inulin would not determine the total inulin present if this method were used. In studies involving the simultaneous determination of the total body inulin space and tissue distribution, it became necessary either to develop a method for tissue analysis which would not destroy the inulin, but, at the same time, would destroy the large inulinoid blank of muscle; or to develop a stable form of inulin for injection into these animals.

Table I indicates the results of treating inulin and muscle with various forms of digestion. From this, it is apparent that some inulin is destroyed by any procedure involving acid, alkali or heating. Inulin probably consists of at least 3 fractions—a heat or dilute acid labile one, a hot alkali labile one, and a hot alkali stable one. This is also suggested by the variation in molecular weight deter-

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TABLE I. Recovery of Usually Available Inulin from Solutions and from Inulin-Muscle Mixtures.

Solution and treatment	% of original inulin remaining after treatment without added tissue	Inulinoid blank of muscle as % of recovered inulin†
1. Water without heat	100%	300%
2. " + heat*	94	200
3. Dilute HCl without heat	95	200
4. " " + heat	92	200
5. " NaOH without heat	85	200
6. " " + heat	66	10

* Heated for 30 min in boiling water bath.

† .30 g of inulin/g of muscle; this corresponds to a plasma level of approximately 200 mg %.

TABLE II. Recovery of Stable Inulin after Treatment with Dilute NaOH and Heat.

.40 mg inulin and muscle added to water	Amt of inulin recovered, mg	% recovered
No muscle	.400, .397, .385	99
1 g muscle	.402, .350, .403, .381	96

minations with heating(2). Furthermore, the only method (No. 6) which satisfactorily reduces the inulinoid blank of muscle, also destroys the most inulin. Therefore, it is necessary to have a form of inulin for injection which will be stable in hot dilute alkali if analysis of tissues such as muscle is desired.

Purified stable inulin is prepared by first heating a 10% solution of chemically pure inulin with concentrated NaOH (5 ml NaOH for 100 ml of inulin solution) in a boiling water bath for 30 minutes. Further heating for 2 hours does not hydrolyze any more inulin. The resultant brown solution is then neutralized to pH 7 with concentrated HCl. The brown color is probably the result of carmelization of the hydrolyzed inulin. The stable inulin is then purified by repeated precipitation with 95% ethyl alcohol. About 5 volumes of alcohol are necessary for the immediate precipitation of one volume of 10% inulin solution. After precipitation, the solution is centrifuged, the supernatant discarded, and the precipitate is redissolved by adding about 75% of the original volume of distilled water and heating in a boiling water bath until solution occurs. The alcohol precipitation is then repeated. From 7 to 10 such precipitations are necessary to produce a pure white powder which gives a clear colorless solution when dissolved in distilled water.

Intraperitoneal injection of this inulin (0.55 g inulin/kg body weight) into weanling rats produced no observable ill effects and did not alter their rate of growth. This material was used for studies in nephrectomized dogs (0.25 g/kg body weight)(3) without any adverse effects. Typical recovery experiments are shown in Table II. Within the reproducibility of results possible with this method (±4%), we feel this represents complete recovery.

We have modified the method of Ross and Mokotoff(1) in two respects: first, by making our final volume, prior to digestion, to 35 ml instead of 50 ml in order to analyze smaller samples of tissue; second, in short-term experiments, by taking a specimen of tissue before the injection of inulin, for the blank determination in order to be able to treat the blank tissue and the experimental one in the same way.

Tissues such as skin and tendon which have only a small amount of inulinoid material in the blank, can be analyzed by extracting the tissue with a known amount of distilled water, after previously mincing the tissue. However, unless this mincing is done very carefully, the hot alkaline digesting method gives more consistent results.

Summary. 1. Current methods for the determination of inulin in tissues are not applicable for simultaneous studies of total body and individual tissue distribution of this substance. A form of inulin stable in hot alkali is required. 2. A procedure for the preparation of a purified form of inulin which is stable in hot, dilute NaOH is presented. 3. Toxicity and recovery experiments indicate this new form of inulin is satisfactory for the direct determination of the inulin content of the tissues of experimental animals following intravenous injection.

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