simple differences in the structures of aminoacids caused changes in the rates of hydrolyses of simple esters not dependent upon the hydrogen ion concentration alone; in other words, that they act selectively on the esters. These actions, it is true, are comparatively small, but, if simple aminoacids can be shown to duplicate, even to less degree, the actions of different lipases, it is not only possible, but probable, that groupings in complex proteins exist and may be found which will account for the enzymatic changes. There is no direct evidence that such groupings are the active factors, but the indirect evidence shows that the existence of such groupings is probable and would be sufficient to account for the reactions.

The writers wish to thank Mr. Morris Erenstoft for his efficient aid in carrying out the experimental work described in this paper.

Summary.

The extraction results described in a former paper were confirmed with a new castor bean preparation.

The action of the castor bean preparation on triacetin in the presence of some neutral salts was described. The activity of the preparation was tested after drying and heating under different conditions.

An esterase preparation, active toward ethyl butyrate, was separated by extraction with water, and its properties studied in solution and in the solid form. Its probable identity with glycerophosphatase was suggested.

A lipase preparation, active toward triacetin, was separated by extraction with 1.5 N sodium chloride solution, and its properties studied.

The forms of combination of the nitrogen in the preparations were determined. The probable protein nature of the esterase and lipase was discussed.

New York, N. Y.

THE TOTAL AMINO NITROGEN IN THE SEEDLINGS OF THE ALASKA PEA.

By Thos. G. Thompson.[1]

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While considerable attention appears to have been devoted to the occurrence of asparagin in plants, very little study seems to have been given to the total amount of amino and amide nitrogen that exists in plants at various stages of growth. Schulze[2] published his ideas concerning the formation of amino acids in plants in 1898. Later Scruti[3] pointed out the importance played by phosphorus in the formation of acid amides and amino acids which go to build up the protein molecules. It is a gener-

[1] Acknowledgment is made to Dr. G. F. White who suggested and supervised this research.

[2] *Z. chim. Physiol.*, **24**, 1898.

[3] *Staz. sper. agrag. Ital.*, **41**, 456.

ally accepted fact that the existence of these nitrogenous compounds is a result both of the decomposition of the plant proteins and of synthetic processes. The exact steps of the syntheses are not definitely known. Franzen[1] has worked out a long series of reactions explaining, from a purely theoretical standpoint, the synthetic formation of the amino acids and the acid amides. His assumptions are based entirely upon the theory of Treub, in that the formaldehyde unites with the ammonia and the hydrocyanic acid to form the amino nitriles, which are later subjected to hydrolysis. The formaldehyde is formed by the reduction of the carbonic acid, and the ammonia and hydrocyanic acid are considered as the reduction products of the nitrates. From the theoretical explanation of Franzen and the experimental work of several investigators, it is concluded that the nitrogenous matter in the plants is constantly undergoing changes. Thus the percentage of the amino nitrogen should vary at different stages in the growth of the plant.

In 1890, Frank called attention to the fact that the leaves of plants contain more asparagin in the evening than in the morning. Kiesel[2] much later made a study of the changes undergone by the nitrogenous matter in *Trifolium Pratense Rept.* When the plants were kept in the dark he was able to demonstrate the presence of a small amount of arginine and showed the absence of other hexone bases. In the fresh plants, those that had been influenced by the action of sunlight, he was able to detect slight traces of amino valeric acid and leucine with more or less certainty. Upon keeping the plants in the dark for some time, however, enough leucine was present so that it was identified without any difficulty.

Andre[3] studied the percentage of amino and amide nitrogen in the nitrogenous materials found in the leaves of plants at different stages of their growth during the year. The leaves in active life, during the months from May to October, upon subjection to analysis, indicated that the total nitrogen (dry material) decreased from 3.4% to 1.8%, while the amino nitrogen at first decreased from 0.53% in May to 0.10% in July, and then gradually increased again to 0.48% in October. As the result of his work, he concludes that the elaboration of the amide nitrogen may be considered uniform throughout the life of the leaf, with the exception of the period of fertilization of the flowers. At this period, translocation of the nitrogenous matter in the plant became very marked.

Experimental.

This investigation was undertaken to obtain some systematic data concerning the relation of the total nitrogen to the amino nitrogen in the grow-

[1] *Chem. Zentr.*, **1910**, II, 983.

[2] *Z. physiol. Chem.*, **49**, 72–80 (1906).

[3] *Compt. rend.*, **148**, 1685–1687.

ing plant, applying a method for the determination of the amino nitrogen which would prove to be, at the same time, both accurate and simple. The method used in the determination of the amino nitrogen in this research consists in the measurement of the nitrogen evolved according to the general reaction:

$$RNH_2 + HNO_2 = ROH + N_2 + H_2O$$

This reaction was permitted to take place in the apparatus recommended and fully described by D. D. Van Slyke[1] for the determination of small percentages of amino nitrogen. This apparatus was utilized exclusively in obtaining the percentages given below. In brief, it consists of a deaminizing vessel which is connected with a buret of 2 cc. capacity and a tube through which acetic acid and sodium nitrite are permitted to flow into the deaminizing vessel. A small buret is used to introduce the required amount of sample, containing the amino acids, into contact with the solution of the nitrous acid. The deaminizing portion of the apparatus is also connected to a gas buret that is graduated to 0.02 cc. This in turn is joined to a Hempel pipet containing an alkaline solution of potassium permanganate for the absorption of the nitric oxide, which is liberated in considerable quantities during the reaction. The detailed manipulation of the apparatus is completely described by Van Slyke in both of his papers. The modified form of the apparatus was used in making all the analyses reported in this paper.

For the purpose of determining the total nitrogen present in the seedlings, the standard Kjeldahl method was utilized.

In running blank determinations with the amino apparatus, a small volume of gas that was not absorbed by the alkaline potassium permanganate was obtained on using sodium nitrite that had been permitted to stand for a day and then filtered from the slight amount of sediment. After running a number of blank determinations and securing their readings it was found that a correction of 0.188 mg. of nitrogen would have to be made. On the other hand, if sodium nitrite was employed that had not been filtered, it was found that a greater amount of gas remained unabsorbed, and thus a correction of 0.199 mg. of nitrogen would be necessary in the regular determinations. In all the experimental work given in this paper, only the filtered sodium nitrite was employed.

From one and a half to five grams of the samples of the seedlings, or different portions of the plant for analysis, were weighed out, transferred and ground finely in a mortar. Water was then added to dissolve the amino acids that were present. As some of the proteins are more or less soluble in water, a drop of glacial acetic acid was introduced into the solution in order to precipitate those that might be present. The solutions were made up in graduated flasks of 25 cc., 50 cc., or 100 cc. capacity,

[1] *J. Biochem.*, **9**, 185 (1911); **12**, 275–284 (1912).

depending upon the size of the sample taken for analysis. Before running into the small buret, attached to the deaminizing vessel, the solutions were carefully filtered.

The formation and distribution of the amino acids and the acid amides in the Alaska pea were studied, special attention being given to its occurrence in the very young plants or seedlings. All the specimens for analysis were grown in a rich, moist soil and in a room of moderate temperature with full advantages for securing the rays of the sun. Every twenty-four hours a few of the seedlings were removed from the soil and, after removing all the earthy particles carefully with the aid of a soft brush, subjected to analysis. This was done for seven consecutive days with the following results:

TABLE I.

	Natural.		Dry material		
	Total nitrogen. Per cent.	Amino nitrogen. Per cent.	Total nitrogen. Per cent.	Amino nitrogen. Per cent.	Amino N. / Total N Per cent.
1-Day seedlings	1.75	0.115	3.27	0.215	6.58
2-Day seedlings	1.51	0.124	3.22	0.265	8.23
3-Day seedlings	1.47	0.151	3.28	0.337	10.52
4-Day seedlings	1.42	0.164	3.35	0.386	11.52
5-Day seedlings	1.067	0.197	3.44	0.635	18.46
6-Day seedlings	0.997	0.214	3.48	0.747	21.46
7-Day seedlings	0.934	0.262	3.53	0.998	28.27

The original seed contained 0.088% of amino nitrogen.

In all cases, samples of the fresh plant were used for the exact estimation of both the total and the amino nitrogen. A third sample, for the determination of moisture, was always weighed out and dried in a closet at the temperature of boiling water for five or six hours. From the results given above, it is seen that there is a steady increase in the percentage of the amino nitrogen and that there is a marked increase in the occurrence of the amino nitrogen in the first- and the fifth-day seedlings. This increase in the first-day seedling is due, undoubtedly, to the decomposition of the proteins present in the original seed and indicates the formation of the food supply for the new protein material. The one-, two- and three-day seedlings all maintained the shape of the seed, but were, of course, much larger in size. The four-day seedling contained a small plumule, while the five-day seedling took the form of a regular plant. The plumules and roots of the six- and seven-day seedlings were larger and showed more signs of development. The large increase in the percentage of amino nitrogen in the five-day plant is probably explained by the rapid transformation of the seed and thus the requirement for a greater quantity of plant food of this nature to build up the highly elaborated nitrogenous material in the leaves, stems and roots. As the amino nitrogen increases at a much greater rate than the total nitrogen, there must necessarily be a marked breaking down and decomposition of the proteins present in the original seed. This fact is also further emphasized in that there is a decided increase of amino nitrogen when expressed as the percentage of total nitrogen.

From the figures given in the foregoing table, it is seen that when the percentage of the total nitrogen decreases as indicated by the results obtained from the seedlings in their natural condition, the percentage of amino nitrogen increases. When this data is expressed in terms of dry material there is a slight increase in the total nitrogen and a very decided increase in the amino nitrogen.

In the case of the seven-day seedlings, the plumules, cotyledons and roots were analyzed separately and the following results were obtained:

Table II.

	Natural.		Dry material.		
	Total nitrogen. Per cent.	Amino nitrogen. Per cent.	Total nitrogen. Per cent.	Amino nitrogen. Per cent.	Amino nitro./Total nitro. Per cent.
Plumule	0.832	0.301	7.021	2.540	36.18
Cotyledon	1.534	0.202	3.36	0.421	12.53
Root	...	...	5.33	1.705	31.99

From the above it is seen that the plumule contains a much greater amount of amino nitrogen, which undoubtedly serves as a food supply for the newly forming leaves and stems. It was expected that the cotyledon would show a greater percentage of amino nitrogen, owing to the decomposition of the protein material that it contained. However, this was not the case, and it is probable that any amino acids that are formed there are translocated to the roots and the stems. This is further indicated by the fact that the percentages of the amino nitrogen in the plumule and the root are much greater than in the cotyledon.

The leaves, the stems near the top, and those near the roots of the plant, were subjected to analysis in the fourteen-, twenty-one and thirty-eight-day plant with the following results:

Table III.

	Natural.		Dry material.		
	Total nitrogen. Per cent.	Amino nitrogen. Per cent.	Total nitrogen. Per cent.	Amino nitrogen. Per cent.	Amino nitro./Total nitro. Per cent.
14-Day leaves	1.52	0.297	9.43	1.830	19.41
21-Day leaves	1.52	0.244	9.29	1.495	16.09
38-Day leaves	1.46	0.209	8.35	1.195	14.32
14-Day stem (top)	0.745	0.303	7.801	3.173	40.67
21-Day stem (top)	...	0.283	...	2.975	...
38-Day stem (top)	0.807	0.226	8.54	2.390	27.99
14-Day stem (root)	0.693	0.300	8.59	3.717	43.30
21-Day stem (root)	0.688	0.275	8.75	3.500	40.00
38-Day stem (root)	0.675	0.187	8.54	2.364	27.68

The total nitrogen remains fairly constant in the natural leaves, but there is a noticeable decrease in the amino nitrogen. When these results are expressed in terms of the dry material, it is seen that there is a decrease in both the total and amino nitrogen as the plant increases in age. The same may be said of the stems both near the top and the bottom of the plant. It is also noticed that the amino nitrogen in the stems near the lower part of the plant are just a little less in percentage than the sample taken near the leaves.

In the case of these older plants it was impossible to get good, reliable results from the analysis of the roots because of the great difficulty of cleaning and freeing them from foreign materials. The results that were actually obtained from the analysis of the roots seemed to indicate a smaller percentage of amino nitrogen than was obtained in the stems. It also seems probable that the extreme ends of the roots contained a much smaller amount of amino nitrogen than the upper of the root.

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NEW BOOKS.

Landolt-Börnstein Physikalisch-Chemische Tabellen. Fourth edition, revised and enlarged. Prepared under the direction of Dr. Richard Börnstein and Dr. Walther A. Roth. Berlin: Julius Springer. 1912. pp. xvi + 1310. Price, M. 59.

The greatest shortcoming of the previous editions of this indispensable work has been the entire lack of tables of certain important and much used data. The removal of this deficiency is responsible for the greater part of the increase in size of this edition over the previous one. Among the new tables the following may be mentioned: Homogeneous Gas Equilibria, Equilibria between Organic Substances, Distribution Coefficients, Ionization Constants, Conductivity Data for Non-aqueous Solutions, Hydrolysis Data, Radioactive Constants, and an extended table giving the chief physical properties of all of the more important organic compounds.

E. W. Washburn.

Notes on Elementary Inorganic Chemistry. By F. H. Jeffery. Cambridge University Press. G. P. Putnam's Sons: New York. i + 55 pp. Price, 60 cents.

The purpose of the author of this small volume has been to present "short summaries of certain facts and reactions which are commonly included in a course on elementary inorganic chemistry." It is intended that the book be used in connection with lecture and laboratory work in order that the notes presented may "serve for revision purposes" and also that they may "afford the comparative beginner groups of facts which he might find difficult to coördinate for himself." Among the ten chapter headings are found such topics as: Acids, Bases and Salts, Oxidation and Reduction, The Action of Certain Acids on Some Metals, and Certain