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BIOSYNTHESIS OF AMINO ACIDS ACCORDING TO SOIL FERTILITY

I. TRYPTOPHANE IN FORAGE CROPS [1] *)

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Our complete dependence on plants for their synthesis of many amino acids calls for our best efforts to explain the biosynthesis of these vital substances. The two fundamental concepts, namely, (*a*) that proteins are composed of amino acids, and (*b*) that the construction of these protein particles occurs only when all the requisite building stones are present, includes the major share of our knowledge about protein synthesis. Any elucidation of the mechanism by which the amino acids are formed would seemingly call for study of the essentially and of the functions of the inorganic elements catalizing these synthese reactions in plants and other life forms.

The service of the various soils, as they may, or may not, deliver adequate supplies of the inorganic nutrient elements, emphasizes further the need for studies integrating the elements of soil fertility with the resulting organic chemical pattern within feed or food crops. Here, then, is the suggestion of the basic fact that our nutrition, and resulting health, ultimately depend upon the soil and the climatic forces that determine it. The development of the divergent soils from the various rocks according to rainfall, temperature, and other meteorological components of climate has definitely established the control of the soil by the climate. We can then reason that through its inorganic contribution to the plant, the soil controls the nutritive quality of food. In turn, this quality of food controls our health.

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The leaf, rather than the stem of the green plant provides a biosynthetic factory wherein we may view the diversity in the array of the organic products [5]). The materials entering the plant from its environment are, for the most part, inorganic elements of the most simple character. By proper manipulation of these substances the plant can build a large variety of molecules of widely differing degrees of complexity.

Previous studies. Protein synthesis, conceivably, begins with the transformation of the carbon chain. The companionship of magnesium and phosphorus in the glycolytic system assumes added importance when viewed in this new light of synthesis. Calcium and potassium interplay for the accompanying reduction of the nitrate. Iron, magnesium, copper, molybdenum, and boron all exert their respective influences. Zinc has been shown to be required for tryptophane synthesis [11]). The rather extensive literature on protein synthesis has been reviewed at different times by McKee [2]), Nightingale [3]), Chibnall [1]), and Wilson [12]). Bjorksten believes that amino acid synthesis involves the condensation of the enol form of pyruvic acid with an acid amide. The resulting product breaks down to form an organic acid and aminoacrylic acid. The latter condenses with other compounds. Petrie [4]) believes that in plants the tricarboxylic acid cycle may be operative in the production of succinic and α-ketoglutaric acids. These may be converted into their respective amino acids by transamination, and serve to store excess nitrogen in the form of their amides, asparagine and glutamine. The primary question involves the reduction of nitrates to nitrites and ammonia. This reaction seems to be coupled with the oxidation of carbohydrates.

That a metabolic block, which would increase the content of free acids, including the amino, might occur when the soil-borne elements were not present in sufficient amounts, has recently been shown by the work of Steinberg, Bowling and McMurtey [7]). They indicate that free amino acids in leaves of plants showing symptoms of low mineral supply increased with nitrogen deficiency by 32%; phosphorus 48%; potassium 58%; calcium 120%; magnesium 283%; and boron 27%. Tisdale *at al.* [10]) reported the methionine and cystine contents of two strains of alfalfa indicating that the sulfate in the substrate influenced the concentration of

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these two organic acids in the alfalfa forage. The authors ⁶) showed that optimum concentration of sulfate for methionine production by alfalfa was 64 p.p.m. and by soybeans 96 p.p.m. They further indicated that the application of 200 pounds per acre of Flowers of Sulfur to a planosol soil of the Southwest Missouri prairie region almost doubled the concentration of methionine in the dry Sudan grass. The work reported herewith is a similar study in which there was determined the tryptophane concentration in various forages grown on various cultural media in the greenhouse.

Experimental Methods. The experimental approach consisted of the critical assays for tryptophane in plant samples grown in both solution and colloidal clay cultures. Soybeans and alfalfa were grown on white quartz sand offered the nutrient solution given in Table I.

TABLE I

Nutrient solution used in quartz sand cultures

Element	Full Nutrient (p.p.m.)
K	547.00
Ca	400.00
Mg	96.00
N	24.80
P	124.00
S	128.00
Fe	34.06
Mn	1.10
B	1.08

Magnesium, manganese, boron, and iron were withheld as individual omissions only. Soybeans were also grown on electrodialyzed colloidal clay and certain silt mineral mixtures. The treatments and results for these are given in Table II. Redtop was grown on colloidal cultures with high, medium, and low calcium each coupled separately with high and medium phosphorus.

Random samples of the plants produced under these environmental conditions were harvested, dried at 70°C, ground to pass through a 60-mesh sieve and stored in air-tight glass containers for assay in the laboratory. The tryptophane was determined on the hydrolyzed plant materials by the microbiological procedure of

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TABLE II

Concentration of tryptophane in soybean hay grown on colloidal Putnam clay and magnesium mineral mixtures *)

Culture Composition	Tryptophane mg/g	Total Nitrogen %
10 g Wyomingite, 50 g H-clay, and 0.5 mmols. H_3PO_4	0.47—0.50	1.94
10 g Glauconitic dolomite, 50 g H-clay, and 0.5 mmols. H_3PO_4	0.66—0.90	1.40
50 g H-clay, 25 m.e. Ca, 10 m.e.Mg, and 0.5 mmols. H_3PO_4	1.06—1.09	1.56

*) These plants grown in greenhouse culture by H. C. Turley.

Stokes and Gunness 8) 9) using *Lactobacillus arabinosus* 17–5 as the assay microbe.

Results. Indol-3-acetic acid has frequently been associated with the boron supply from the soil. There is the suggestion in the data in Table III of a possible relation between this element and the concentration of the indol amino acid-tryptophane-in both the alfalfa and soybean hays. Even though the enzymatic function of boron remains quite obscure, these data substantiate the conclusions of Briggs, namely, that an interruption in the synthetic processes just prior to protein formation occurs when boron is in low supply.

TABLE III

Concentration of tryptophane in alfalfa and soybean hays according to amounts of boron offered in the nutrient solutions

Soybean Hay		Alfalfa Hay	
Boron Offered p.p.m.	Tryptophane mg/g	Boron Offered p.p.m.	Tryptophane mg/g
0	1.38	0	1.27
0.27	1.89	0.22	1.36
1.28	3.10	0.44	2.17
		1.08	2.55

In an effort to determine the magnitude of tryptophane synthesis when the elements commonly supplied by the soil are in low amounts magnesium, boron, manganese, iron, calcium, and phosphorus were each withheld separately from the growth medium. The results of these modifications of the plant environment, recorded in Table IV, show the pronounced variation in the concentration of this essential amino acid in soybeans and alfalfa as legumes and in redtop, a grass,

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TABLE IV

Concentrations of tryptophane in alfalfa, soybean, and redtop hays according to inorganic nutrients precent in the substrate

Plant	Treatment	Tryptophane mg/g
Soybeans	Mg withheld	1.80
Soybeans	B withheld	1.89
Soybeans	Full nutrient	3.10
Soybeans	Mn withheld	1.93
Soybeans	B withheld	0.57
Soybeans	Fe withheld	1.76
Soybeans	Full nutrient	2.10
Soybeans	Wyomingite	0.47—0.50
Soybeans	Glauconitic dolomite	0.66—0.90
Soybeans	Exch. Mg. on clay	1.06—1.09
Alfalfa	Mn withheld	1.47
Alfalfa	B withheld	1.15
Alfalfa	S withheld	2.64
Alfalfa	Fe withheld	1.74
Alfalfa	Full nutrient	2.80
Redtop *)	High P, High Ca	2.65
Redtop	High P, Medium Ca	2.21
Redtop	High P, Low Ca	2.09
Redtop	Medium P, High Ca	2.38
Redtop	Medium P, Medium Ca	1.88
Redtop	Medium P, Low Ca	1.38

*) Grown on colloidal clay culture.

according to the corresponding shortage of these elements of soil fertility. Where magnesium was withheld from soybeans grown in solution, the tryptophane content was reduced from 3.1 to 1.8 milligrams per gram of dry forage. In studies of some magnesium minerals from which this element could be weathered at varying rates by the acid colloidal clay, the tryptophane content was lowered in proportion to the magnesium made avalable from that reaction. The concentration of this amino acid in the case where Wyomingite was offered was only one-half of the value of that where the magnesium was readily exchangeable on the clay as shown in Table II. Magnesium from the dolomite was more available and expressed itself through increased synthesis of tryptophane by the plants. These data carry the suggestion that either less carbohydrate was

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built by photosynthesis or less was respired to yield a critical linkage in the tryptophane molecule. The enzymatic formation of the indol ring could require magnesium as the activating cation at some stage of its synthesis.

Where boron was withheld, the tryptophane in the forage was always substantially lower. When manganese was omitted from the solution offered these alfalfa plants, the concentration of this amino acid in the hay was reduced 50% below that from the full nutrient solution. When sulfur was not supplied little if any reduction in tryptophane formation occurred. Both calcium and phosphorus seemingly promote production of this essential amino acid, if we are to judge from the data for the redtop in Table IV. This grass was grown on two levels of phosphorus each coupled on two levels of phosphorus each coupled with high, medium, and low applications of calcium. On both levels of phosphorus, the tryptophane in the forage was increasingly higher with increasing concentration of calcium. The tryptophane values for the plants receiving high phosphorus treatments were significantly above those given medium amounts of phosphorus. While the function of the elements in tryptophane synthesis is far from clear, general considerations point to the part these ions might play. Phopshate, for example, is the key ion in intermediate metabolism. It must be supplied by the soil in quantities commensurate with the needs of these carbohydrate transformations. Phosphate serves not only to supply sufficient energy to maintain the living state, but also to aid in the biosynthesis of such molecules as may be precursory to tryptophane. Also, we have the suggestion that at least zinc, boron, manganese, and now calcium, phosphorus, and magnesium are involved. In view of the many roles of these soil-borne nutrients in the intermediate metabolic pool, precise study of these enzymatic relations is strongly indicated for the future work.

Discussion. In the physiology of an organism we have regularly recognized the necessity of considering the inorganic environment in attempts to explain any performance by it. Yet the soil, as the source of nearly all the required nutrient elements remains much of an unknown. The measurement of the delivery of the inorganic elements in the composition of the burnt plant material, as the only effect of the soil, has been an unfortunate, though prevalent, criterion of the contribution by the soil factor of the environment. This little appre-

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ciation of the soil has persisted even when the ecological array of plant species suggests that plants must be of diverse organic quality as they evolve on the diverse soils.

Consideration of the physiological roles of the inorganic nutrients has led us in this study to picture them active in the enzymatic mechanisms of biosynthesis. This process, in which the simple carbohydrate is converted into the protoplasmic protein by the help of the soil-borne elements, demands a more detailed study of the plant enzymes containing these inorganic nutriens. The biocatalysts performing in the construction of the protein include those functioning in the intermediate metabolic pool. The key to amino acid synthesis would seem to lie in a study of enzymes containing, or activated by, the soil-borne nutrients wherein these biocatalysts enable the formation of the residual structures precursory to each amino acid molecule.

Visual symptoms of plant deficiency need not be evident to prove deficiencies in the health, or vigor, of the organism. Deficiency is a matter of degree. Plants, like animals, may become decidedly inferior in quality long before showing outward signs of such. It is in this broad range between optimum and deficiency that such biochemical assays of quality assume greater importance. It would seem that any biological assay of soil fertility must ultimately measure molecules as the reactants in biosynthesis, and not merely the delivery of ash in the forage. The whole biotic pyramid may well be viewed as the simple combinations of colloidal particles according to enzymatic specificity to yield the vital cellular constituents according to the fertility of the soil.

Summary

A microbiological assay for tryptophane, as a required amino acid for the diet of the white rat, in alfalfa (*Medicago sativa*), soybean (*Soja max*), and redtop (*Agrostis stolonifera major*) hays, showed that this organic substance of particular food value varied widely according to the inorganic composition of the substrate upon which the plants synthesizing it were grown. The formation of tryptophane was found to be proportional to the available boron when this anion was the limiting element in the culture solution. Tryptophane synthesis was decreased when magnesium, boron, manganese, and iron were withheld from solutions offered alfalfa and soybeans. Increasing the calcium increased the production of this amino acid. The enzymatic pattern of biosynthesis revealed itself pointing out some

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possible reasons why such diversity in the components of the plant protein might be expected. The effects of the inorganic nutrient elements were shown to be of the same kind whether the plants were grown in nutrient solution or in colloidal clay.

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