Growth studies and gas exchange measurements have revealed differences in the capabilities of the mutant strains. It is probable that different reactions are blocked in each. By determining which reactions are blocked in these mutants, together with similar studies on others, it is possible that a number of intermediate steps in the photosynthetic mechanism can be positively determined.

A more complete report including supporting data will be published at a later date.

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Fractionation of Amino Acids From Hydrolysates in Nonaqueous Systems

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All of the amino acids which are present in an acid hydrolysate of a protein can be brought into solution in acetone by the action of certain organic acids which form with them salts or complexes which are soluble in this solvent. Thus far we have found no other solvent as good as acetone for this purpose. Alcohols react with the reagents, and dioxane, although of possible usefulness in special cases, is less satisfactory than acetone. Methyl-ethyl ketone is greatly inferior to acetone as a solvent for the amino acids with the organic acids which we have tested as reagents. We have explored the possibilities of fractionation of amino acid mixtures in several nonaqueous systems, making use of differences in solubility of individual amino acids in acetone solutions of certain organic acids.

A large number of acids have been examined for their power to cause solution of amino acids in acetone. The following possess this property to a useful degree: trichloroacetic acid, dichloroacetic acid, *p*-toluenesulfonic acid, 4-nitrochlorbenzenesulfonic acid, *d*-camphorsulfonic acid, *dl*-camphorsulfonic acid, benzenesulfonic acid, and *n*-butylsulfonic acid.

The ammonium salts of these acids are soluble in acetone. On addition of dry ammonia gas, the amino acids are precipitated from such solutions when trichloroacetic acid, benzenesulfonic acid, or *dl*-camphorsulfonic acid is employed, the basic amino acids being exceptions. Arginine, histidine, and lysine separate in combination with the reagent acid. When *p*-toluenesulfonic acid, 4-nitrochlorbenzenesulfonic acid, or *d*-camphorsulfonic acid is employed as reagent, the amino acids separate in great measure in the form of complexes when dry ammonia gas is introduced in excess.

When an acid hydrolysate of casein is dissolved in acetone with the aid of trichloroacetic acid, benzenesulfonic acid, or *dl*-camphorsulfonic acid, and an excess of ammonia is introduced, about 88% by weight of the sample is recovered in the resulting precipitate. In the case of individual amino acids brought into solution in this way, recovery on precipitation is not far from 100%.

Table 1 shows the molecular ratios between reagent acid and amino acid necessary to bring the latter into solution in acetone when the concentration of acid in acetone is as indicated.

TABLE 1

Amino acid	Moles of acid reagent / Mole of amino acid		
	Trichloroacetic acid*	*dl*-Camphorsulfonic acid†	Benzenesulfonic acid‡
Proline	4.5	1.1	1.0
Threonine	5.5	1.4	1.6
Tyrosine	68.0	1.4	1.4
Isoleucine	5.1	1.0	1.6
Alanine	5.0	1.6	1.8
Valine	5.1	1.4	1.8
Aspartic acid	53.0	1.6	2.0
Phenylalanine	5.2	0.9	3.2
Serine	20.0	0.8	3.4
Hydroxyproline	25.0	2.1	3.6
Leucine	5.4	1.6	4.2
Norleucine	5.4	1.3	7.4
Tryptophan	3.0	28.6	10.6
Cystine	Insol.	10.0	13.4
Glycine	4.2	> 22.5	16.2
Glutamic acid	55.0	1.7	44.0
Methionine	6.2	0.9	64.0
Histidine	6.8	3.7	128.0

* The trichloroacetic acid in acetone was a 0.24 N solution.

† The *dl*-camphorsulfonic acid in acetone was a 0.2 N solution.

‡ The benzenesulfonic acid in acetone was a 0.4 N solution.

Fractionation of hydrolysates or other mixtures of amino acids has been accomplished by the following procedures:

(1) Fractional solution: Increments of reagent (acid in acetone) are brought successively into contact with dry hydrolysate or other mixture of amino acids, in the finely ground state, contained in a filter funnel or crucible. After brief contact the reagent, containing some dissolved amino acids, is removed by applying suction. The receiver is changed, and another increment of reagent is applied to the undissolved portion of the hydrolysate, and this is then removed by suction. In this way a hydrolysate can be separated within 2 or 3 hrs into as many as 50 or 60 fractions.

(2) The hydrolysate can be completely dissolved in the minimum amount of the acid-acetone reagent, and fractions of amino acids can then be dropped out by the stepwise introduction of dry ammonia to precipitate successive fractions of the dissolved amino acids. Ammonia can be introduced in the form of a strong solution

in acetone, but this leads to a change in relation of acetone to reagent.

(3) The hydrolysate in solution may be separated into fractions by the stepwise introduction of hydrogen chloride, either directly as the gas or by means of a strong solution of hydrogen chloride in acetone.

(4) Fractionation of solutions can be brought about by successive additions to the acetone-reagent-amino acid system of suitable amounts of some solvent which is miscible with acetone but which does not have a solvent action on the amino acid-reagent complexes.

(5) Trichloroacetic acid solutions of amino acids in acetone undergo spontaneous precipitation owing to the fact that amino acids catalytically decompose trichloroacetic acid into chloroform and carbon dioxide. As the acid is decomposed, the amino acids progressively precipitate. Chromatographic analysis of fractions obtained in this way show that individual amino acids do not drop out successively but that several come out together. There is, however, in this procedure a method for obtaining greatly simplified amino acid mixtures as compared with the hydrolysate from which the fractions are obtained.

Fractions of amino acids obtained by the above-described principles from hydrolysates of proteins have been submitted to examination by paper chromatography according to the method described by Consden and his associates (*1*). The results show that from hydrolysates containing 18 or more amino acids the components of a series of 20–60 successive fractions obtained by method 1 differ in their qualitative composition. Those amino acids most readily soluble in the reagent employed tend to be most abundant in the earlier, and the least soluble ones tend to accumulate in the later, fractions.

When such fractions are rechromatographed at successive dilutions, one after another of the constituent amino acids fails to appear on the developed chromatogram. By this means it is possible to secure an approximate appraisal of the quantitative composition of any fraction with respect to its component amino acids. Each amino acid in a fraction can be identified by chromatographing the fraction in parallel with seedings of pure amino acids. The known keeps step with the unknown and reveals its identity.

Application of the available color reactions for individual amino acids to the fractions obtained by the methods described reveals that these amino acids are not present throughout the series but are found only over limited ranges of the successive fractions.

Extensive fractionation of a hydrolysate, identification and approximate quantitative analysis of successive fractions, provide information as to the similarity of fractions. This may then be used as a basis for the recombination of similar fractions in order to reduce the number of fractions to be subsequently worked with in further separation of the components. In the case of certain fractions it has been found that refractionation with the same reagent may lead to further simplification of the composition of the mixtures of amino acids which they contain. But, as will be seen from the accompanying table, advantageous procedures for isolation of individual amino acids are available by changing to a second reagent for further fractionation.

We are pursuing our investigation of the possibilities of applying the principles here described to the isolation of individual amino acids from partially and completely hydrolyzed preparations from proteins. Our studies, details of which will form the subjects of later communications, have indicated the practicability of these methods.

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The Control of Grass Weeds in Sugar-Cane Fields in Puerto Rico

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In the course of experiments and field tests conducted during the last three years with herbicides for the control of weeds infesting sugar-cane lands in Puerto Rico it was soon found that the grass weed population increased when the broad-leaved plants like "cohitre" (*Commelina logicaulis* Jacq.), *Amaranthus spinosus* L., and "bejuco de puerco" (*Ipomoea* spp.) were destroyed by 2,4-D or other herbicides. The need arose for an effective grass herbicide complementary to 2,4-D or for a substitute which might control both broad-leaved and grass weeds.

It has been generally recognized that 2,4-D has little, if any, effect on grasses. Recently, Mangual (*2*) reported that the addition of 2,4-D increased the herbicidal action of oil emulsion (pentachlorophenol in diesel oil) and Concentrate 40 in grass control. Crafts (*1*) recommends an oil emulsion contact spray (pentachlorophenol in aromatic oil) for the control of young grass seedlings and claims that the addition of 2,4-D to the spray further adds to its value, providing a lethal agent for the weeds easily controlled by 2,4-D.

The results of experiments conducted on various soil types with ample replications agree with the above on the value of pentachlorophenol and the combined 2,4-D-pentachlorophenol in the control of grasses. We have further found that 2,4-D in oil provides a more effective grass herbicide than pentachlorophenol emulsion or Concentrate 40.

In a typical experiment on fallow ground and repeated three times, plots were treated with an aqueous solution of 0.1% 2.4-D to kill most of the broad-leaved plants, allowing the grass weeds to develop without competition. The most prevalent volunteer weeds on the plots were *Cyperus rotundus* L., Bermuda grass (*Cynodon Dactylon* (L.) Pers.) *Eleusine indica* (L.) Gaertn., *Digitaria sanguinalis* (L.) Scop., and *Eriochloa polystachya* H.B.K. Sixteen plots were measured out, $20' \times 8'$, and a $4'$ section of each was hoed, spaded, and planted to *Trichachne insularis* (L.) Nees, a grass resistant to most chemical herbicides. Four stools of sugar cane were planted in each plot to determine susceptibility or resistance to