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Effect of Proteins on the Diffusion of Amino Acids Through Membranes.

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The concentration of amino acids in nucleated erythrocytes is known to be several times that of the surrounding plasma. The concentration in mammalian liver and muscle, as indicated by determinations on tungstic acid extracts, is 6 to 8 times that of the blood plasma.

As part of an inquiry into the factors that cause this inequality in distribution we have studied the diffusion of amino acids through tubular membranes of cellophane. The progress of dialysis was measured by amino nitrogen determinations on the inner and outer liquids by the manometric method of Van Slyke. The inner liquid consisted of a 1.5% solution of gold label gelatin. The amino acid was contained in the outer liquid in an initial concentration of 0.001 N. Glycine was generally employed. After 15 to 30 hours at a temperature of 20° the experiments were terminated. The outer fluid was analyzed directly, while the inner fluid was first rendered free of protein by treatment with phosphotungstic acid.

Over a wide range of H ion concentration, (pH 2.5-9.5) the equilibrium concentration of amino acid in the outer fluid was found to be over twice as great as that in the inner protein-containing solution. This inequality persisted, undiminished in magnitude, even in the isoelectric zone of the protein. The equilibrium, moreover, could be approached from the other end, that is by dissolving the amino acid in the inner fluid containing the dispersed protein. In neutral solutions aspartic acid and glutamic acid behaved like glycine in the establishment of a high concentration ratio when dialyzed against 1.5% gelatin. Dialyzed solutions of crystal-

line egg albumin (1.5%) and agar-agar (0.38%) affected the diffusion of glycine at pH 5 in similar fashion.

The persistence of this unequal distribution in isoelectric protein solutions demonstrates that the equilibrium is not of the Donnan type.

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Studies on the Combination of Manganese with Certain Amino Acids and Related Compounds.*

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The present work is a continuation of the studies which have been carried out in this laboratory on the combination of proteins, amino acids, and allied compounds with the inorganic elements.[1, 2, 3] This work has now been extended to include the manganous compounds.

The method employed consists in adding the substances to be tested to a solution of manganous chloride. The solution is adjusted to pH 9.25 by means of a borate buffer. The aqueous solution is shaken for 20 minutes with a solution of isonitrosoacetophenone dissolved in chloroform. The color of the chloroform solution is compared in a colorimeter with a chloroform solution obtained in a similar manner except that the test substance is omitted. If the substance tested forms a compound with manganese (*e. g.,* complex ions) such as to decrease the activity of the manganous ions in the aqueous phase, the color of the resulting chloroform solution will be less intense than the standard when both are shaken for a period of 20 minutes. If the manganous compound is dissociated to a greater extent no effect on the color will be observed.

The results show that oxalic, malonic, succinic, and glutaric acids have a decided influence in decreasing the color. The quantitative effect is greatest in the case of oxalic acid and least in the case of glutaric acid. The effect of the addition of aspartic or of glutamic acid is approximately the same as that of the corresponding nitrogen-free acids. The addition of alanine or of sodium chloride was

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