(13) Non-enzymic Transamination between Glycine and Glyoxylate.

By LAURA W. FLEMING and G. W. CROSBIE. (*Department of Biochemistry, The University, Glasgow, W. 2*)

Non-enzymic transamination reactions of the type

$$R'\cdot CH(NH_2)\cdot CO_2H + R\cdot CO\cdot CO_2H \rightleftharpoons R'\cdot CO\cdot CO_2H + R\cdot CH(NH_2)\cdot CO_2H$$

under physiological conditions have been described by Nakada & Weinhouse (1953) for limited cases where R = H. The equilibria of the reactions were completely in favour of glycine formation. No evidence has been obtained for non-enzymic transamination in the case where both R and R′ are alkyl derivatives.

In the course of a study of C_1-unit formation from glycine a facile non-enzymic transamination reaction between [^{14}C]glycine and glyoxylate has been observed. The reaction proceeds smoothly at pH 7 and 37°. The reaction is catalysed by hydroxyl ions and is inhibited at values of pH lower than 6. At pH 7 the reaction is catalysed by copper ions and inhibited by ethylenediaminetetra-acetic acid. The mechanism of the reaction at pH 7 is envisaged as involving a prototropic re-arrangement of the Schiff base glyoxylideneglycine induced by co-ordination complex formation. The significance of these results will be discussed in relation to the present concepts of the role of glyoxylate in the enzymic formation of formate from glycine.

Pyridoxal phosphate has been found to reverse the catalytic effect of copper ions on the non-enzymic transamination reaction between glycine and glyoxylate. This contrasts markedly with its pronounced catalytic influence on the alanine–pyruvate and glutamate–α-oxoglutarate systems. The mechanism of the involvement of pyridoxal phosphate in non-enzymic transamination reactions was discussed.

The glycine–glyoxylate non-enzymic transamination reaction proceeds with a slow disappearance of glycine and glyoxylate. Chromatographic evidence for the formation of β-hydroxyaspartic acid and pyruvate has been obtained. When the transamination reaction is carried out in the presence of pyridoxal phosphate, hydroxypyruvate is formed in addition to β-hydroxyaspartic acid. The mechanism of the formation of these artifacts will be discussed.

Nakada, H. I. & Weinhouse, S. (1953). *J. biol. Chem.* **204**, 831.

(14) Photofixation of Nitrogen and Photoproduction of Hydrogen by Thiosulphate During Bacterial Photosynthesis.

By D. I. ARNON, M. LOSADA, M. NOZAKI and K. TAGAWA. (*Laboratory of Cell Physiology, University of California, Berkeley, U.S.A.*)

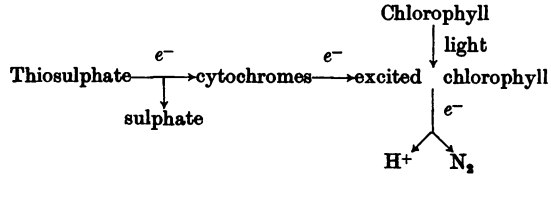
Photosynthetic bacteria fix N_2 or evolve H_2 in light (Kamen & Gest, 1949; Gest & Kamen, 1949). From the standpoint of the electron-flow theory of photosynthesis (Arnon, 1959, 1960), which excludes a photolysis of water, these phenomena could be viewed as resulting from a reduction of N_2 and H^+ respectively, by electrons donated by chlorophyll. The electrons come to chlorophyll via cytochromes from external electron donors and are raised to a reducing potential, at least equal to that of hydrogen gas, by photons absorbed during the primary photochemical act. This theory was tested by supplying thiosulphate as an electron donor to the photosynthetic sulphur bacterium, *Chromatium*.

Thiosulphate was found to reduce the cytochromes of *Chromatium*, that are oxidized by light. Photofixation of N_2 and photoproduction of H_2 were investigated in parallel experiments. Whole cells fixed N_2 with thiosulphate only in the light, and, most vigorously, when oxaloacetate was added as an amino group acceptor. N_2 fixation was inhibited by ammonia (cf. Kamen & Gest, 1949). In the absence of N_2, thiosulphate gave H_2 evolution in light. Gas evolution ceased when light was turned off and resumed when light was turned on again. No CO_2 source was supplied; KOH was present in the reaction vessels. Photoproduction of hydrogen was most vigorous at acid pH, was proportional to thiosulphate concentration (Losada, Nozaki & Arnon, 1960) and was inhibited by N_2 and ammonia.

Similar photofixation of N_2 and photoproduction of H_2 was obtained when thiosulphate was replaced by succinate. With succinate as electron donor, no additional amino group acceptor for photofixation of N_2 was necessary.

Cell-free *Chromatium* preparations fixed N_2 in the dark when supplied with H_2. These findings support the view that the role of light in photofixation of N_2 is to generate electrons with a reducing potential equal to H_2. N_2 fixation in whole cells and cell-free systems was confirmed with ^{15}N.

The following electron-flow mechanism for the photofixation of N_2 and photoproduction of H_2 is consistent with these findings:



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(15) The Effect of Protein Intake on the Ribonucleic Acid of Liver Cell Sap.

By ELIZABETH J. T. PROSSER, ANNETTE C. MALLINSON and H. N. MUNRO. (*Department of Biochemistry, The University, Glasgow, W. 2*)

The metabolism of RNA in the liver is sensitive to changes in the protein content of the diet. From isotopic and other data we have concluded that the rate of breakdown of a part of the liver RNA is regulated by the supply of amino acids from the diet (Clark, Naismith & Munro, 1957). There is evidence that this labile RNA is associated with endoplasmic reticulum and we have suggested that the protein content of the diet alters the relationship between reticulum-attached ribonucleoprotein and cell-sap RNA (Munro & Clark, 1960).

We have now examined the effect of dietary protein intake on the RNA of liver cell sap. This was prepared from rat livers homogenized in medium (Rendi & Campbell, 1959) and centrifuged at 105 000 *g* for 1 hr. The amino acid-activating enzymes and RNA were precipitated at pH 5·2. This fraction was then incubated with [DL-^{14}C]leucine and ATP (Rendi & Campbell, 1959), and the RNA examined for uptake of amino acid. Preparations from three groups of rats were studied (Clark *et al.* 1957). (*a*) Animals undergoing extensive loss of RNA from the cell, as the result of fasting overnight. (*b*) Animals from a similar group fed protein 1 hr. before killing; our previous evidence suggests a sudden cessation of breakdown under these circumstances. (*c*) Animals fed on a protein-deficient diet for several days before fasting overnight. This depletes the labile RNA of the liver cell and there is consequently no further loss of RNA on fasting. The amount of RNA in the cell sap was greatest in group (*a*), in which RNA breakdown was extensive, but it was significantly less active in accepting [^{14}C]leucine. This suggests that the cell-sap RNA was diluted by breakdown products from the reticulum.

The cell sap prepared from these three groups of animals was further fractionated by centrifuging at 105 000 *g* for 3 hr. more, resulting in a precipitate (post microsomal pellet) and a supernatant fluid. Diet no longer influenced the amount or amino acid uptake of the RNA of the supernatant fluid. The amount of RNA in the pellet varied considerably with diet, being greatest in the case of the rats of group (*a*), which were losing RNA from the cell, and least in the depleted animals of group (*c*). When pH 5 fraction was prepared from the pellet, the RNA showed some capacity to accept [^{14}C]leucine; the pellet RNA was most active when prepared from the protein-depleted animals. These findings suggest that the pellet RNA consists of two fractions, one of which varies in amount with diet but is unable to accept amino acids. Further evidence of differences in the heterogeneity of the pellet RNA on different diets was obtained by fractionation on 'Ecteola' columns (Goldthwaite, 1959).

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