Table I. Action of ethionine on the transmethylation between betaine and homocysteine

| Ethionine administration mg/day | No. of rats | μg of methionine formed/h per g of fresh liver |
|---|---|---|
| none | 6 | 377.5 ± 55.8 |
| 5 mg | 6 | 429.2 ± 30.8 |
| 10 mg | 6 | 612.8 ± 87.8 |
| 20 mg | 6 | 503.6 ± 69.9 |
| 40 mg | 6 | 394.0 ± 36.8 |

Table II. Action of homocysteine on the transmethylation between betaine and homocysteine

| Homocysteine administration mg/day | No. of rats | μg of methionine formed/h per g of fresh liver |
|---|---|---|
| none | 6 | 377.5 ± 55.8 |
| 4.12 | 6 | 505.4 ± 94.0 |
| 8.24 | 6 | 618.6 ± 51.3 |
| 16.48 | 9 | 576.4 ± 134.5 |
| 32.96 | 9 | 550.8 ± 142.4 |

*Discussion.* These findings suggest, however, not without some doubt, that the stimulation of betaine-homocysteine-transmethylase activity, induced by ethionine administration, could be attributed to an increased concentration of the acceptor substrate, homocysteine, due to the de-ethylation of ethionine.

On the contrary the stimulation of the enzyme could be attributed to a decreased concentration of the final product of the reaction (methionine), due to an accelerated catabolic degradation of methionine to $CO_2$, induced by ethionine administration[4].

On the other hand, the decreased enzyme stimulation observed with higher doses of L-ethionine is probably in relation to the situation deriving from the fall in ATP concentration in the liver[9] and consequently from the inhibition of RNA[10] and protein synthesis[11].

*Zusammenfassung.* Injektionen von Ethionin oder Homocystein steigern die Transmethylierung in der Leber.

A. PITTONI, F. F. RUBALTELLI and P. L. SUSSI

*Istituto di Chimica Biologica, Università di Padova (Italy), 5 August 1968.*

[9] K. SHULL, J. MCCONOMY, M. VOGT, A. CASTILLO and E. FARBER, J. biol. Chem. *241*, 5060 (1966).
[10] S. VILLA-TREVINO, K. SHULL and E. FARBER, J. biol. Chem. *241*, 4670 (1966).
[11] S. VILLA-TREVINO, K. SHULL and E. FARBER, J. biol. Chem. *238*, 1757 (1963).

## Fixation of Enzyme Protein in Soil by the Clay Mineral Montmorillonite

About 20% of the carbon in soil organic matter is located in amino acids. These amino acids are not in a free state; they are believed to be located in proteins which are fixed in the soil by some unknown mechanism and thus protected against microbial decomposition[1].

Investigations in this laboratory[2-4] utilizing carbon-14-labelled cellulose, hemicellulose and glucose, have shown that a part of the amino acid metabolites formed during the decomposition of added carbohydrates remain in the soils for years. Addition of 2–5% of the clay mineral montmorillonite to the soil samples increased the 'fixation' of amino acid metabolites, and the explanation was advanced that the metabolites fixed by the clay mineral were enzyme proteins excreted during the decomposition of the added material[4]. This assumption has been examined by determinations of the hemicellulase activity of soil samples differently treated with respect to addition of carbohydrates and montmorillonite. The results of this investigation are reported in this article.

The soil used was a sandy soil of pH 5.8, 1.9% organic carbon and 0.18% nitrogen. The montmorillonite originated from Wyoming, the fraction of particles $< 2\ \mu$ was used, it was isolated by sedimentation and saturated with $H^+$ by an ion exchange resin and adjusted to pH 6.0 by addition of 0.5 $N$ NaOH. Details concerning the set up of the experiments, the soil, preparation of the carbon-14-labelled carbohydrates, methods of analysis, etc. are found in references [2] and [4].

The activity of enzymes in soil found outside the living organisms can be measured if the physiological processes can be inhibited without inactivating the enzymes. This can be done either by addition of toluene or by irradiation with ionizing radiation[5]. Both methods have been used in this investigation, samples of 1.5–3.0 g soil containing 14–18% water were placed in screw-capped glass vials of 25 ml capacity, 1 ml of toluene was added to each, or they were given 2.5 Mrad of $\gamma$-radiation from a Cobalt-60 source. 3.0 ml of sterile 0.2 $M$ phosphate buffer of pH 6.5, and 3.0 ml of a sterile 2% aqueous solution of hemicellulose were added to each vial. The content of the vials was thoroughly homogenized and they were placed in a water bath of 40 °C. These conditions have formerly been found by the author to be optimal for determination of hemicellulase activity in soil[6]. Samples were removed from the vials after a suitable time of

[1] J. M. BREMNER, in *Soil Nitrogen* (Ed. W. V. BARTHOLOMEW and F. C. CLARK; American Soc. Agronomy, Madison 1965), p. 93.
[2] L. H. SØRENSEN, Soil Sci. *95*, 45 (1963).
[3] L. H. SØRENSEN, Nature *208*, 97 (1965).
[4] L. H. SØRENSEN, Soil Sci. *104*, 234 (1967).
[5] J. J. SKUJINS, in *Soil Biochemistry* (Ed. A. D. MCLAREN and G. H. PETERSON; Marcel Dekker, New York 1967), p. 371.
[6] L. H. SØRENSEN, Acta agric. scand., Suppl. *1* (1957).

incubation and centrifuged, and the reducing power of the clear supernatant determined by the method of SOMOGYI[7]. The hemicellulase activity of the samples is expressed as mg xylose/ml · g dry soil after 48 h of incubation. The values found in the Table are deducted blank values, the values found in controls where the hemicellulose solution was replaced by water, and corrected for the diluting effect of the water added with the soil samples. The pH of the suspensions was checked after 72 h of incubation, it varied between pH 6.3 and 6.5.

Hemicellulase activity of soil samples in which hemicellulose or glucose were decomposed. Physiological processes in the samples were inhibited by addition of toluene

| Carbohydrate added 500 mg C/ 100 g dry soil | Montmorillonite added % | Period of decomposition days[a] | Added carbohydrate-C recovered in amino acids mg/100 g dry soil[b] | Hemicellulase activity, mg xylose/ml · g dry soil · 48 h |
|---|---|---|---|---|
| Hemicellulose | 0 | 6 | 39.1 | 1.03 |
| | 0 | 12 | 43.3 | 1.00 |
| | 0 | 30 | 40.6 | 0.92 |
| | 0 | 90 | 30.1 | 0.85 |
| | 0 | 700 | 15.7 | 0.18 |
| | 5 | 6 | 58.2 | 2.22 |
| | 5 | 12 | 58.3 | 2.49 |
| | 5 | 30 | 49.6 | 1.93 |
| | 5 | 90 | 49.2 | 1.40 |
| | 5 | 700 | 35.6 | 0.58 |
| Glucose | 0 | 6 | 24.8 | 0.06 |
| | 0 | 12 | 31.8 | 0.06 |
| | 0 | 30 | 36.7 | 0.08 |
| | 0 | 90 | 29.8 | 0.08 |
| | 0 | 300 | 17.7 | 0.07 |
| | 5 | 6 | 77.6 | 0.16 |
| | 5 | 12 | 70.9 | 0.16 |
| | 5 | 30 | 63.2 | 0.12 |
| | 5 | 90 | 52.8 | 0.07 |
| | 5 | 300 | 46.2 | 0.08 |

[a] The soil samples were stored at 20 °C, water was added to 40% of water-holding capacity. 5 mg N in ammonium nitrate were added per 100 mg C added in carbohydrate. $CO_2$ production indicated as mg C/100 g dry soil collected during the first 30 days of decomposition: soil + hemicellulose — montmorillonite 250 mg; soil + hemicellulose + montmorillonite 216 mg; soil + glucose — montmorillonite 400 mg; soil + glucose + montmorillonite 390 mg. [b] Amino acids were released from the soil samples by boiling with 6*N* HCl for 16 h.

It is seen from the Table that addition of 5% montmorillonite to the soil increased the amounts of carbon originally added in hemicellulose or glucose which could be recovered in amino acids. It is further seen that the hemicellulase activity of the soil samples increased with increasing amounts of original hemicellulose carbon in amino acids. The hemicellulase activity of the samples to which glucose was added is very small in all samples and of the same order of size even through the amounts of original glucose carbon in amino acids were approximately doubled as a result of the addition of montmorillonite.

These observations indicate that the amino acid metabolites originating from the added hemicellulose and fixed by the added montmorillonite must be at least partly enzyme protein.

The hemicellulase activities measured in the irradiated samples were 15–25% lower than the values measured in the toluene-treated samples. The difference was largest in the beginning of the decomposition period, the values measured in samples with a decomposition period of 700 or 300 days were almost identical.

The reaction products of the enzyme reaction were determined in a number of the samples by paper chromatography, xylose was the main product, small amounts of arabinose and oligosaccharides were also detected.

*Zusammenfassung.* Aminosäureverbindungen, die im Boden während des biologischen Abbaus von Kohlehydraten gebildet und vom Tonmineral Montmorillonit stabilisiert werden, haben Enzymcharakter und sind folglich Proteine.

L. H. SÖRENSEN

*The Danish Atomic Energy Commission,*
*Research Establishment Risö,*
*Agricultural Research Department,*
*4000 Roskilde (Denmark), 30 July 1968.*

[7] M. SOMOGYI, J. biol. Chem. *160*, 61 (1945).

## Factors Controlling the Chain Length of Fatty Acids Synthesized by the Intestinal Mucosa of Guinea-Pig

Cell-free extracts of lactating rabbit[1] and rat[2] mammary gland have a high rate of fatty acid synthesis from acetate. These extracts can synthesize fatty acids of a wide range of chain lengths, the precise pattern synthesised being greatly influenced by the cofactor conditions used, especially the concentration of malonyl-CoA. This has led to the suggestion that the activity of acetyl-CoA carboxylase (EC 6.4.1.2), the rate-limiting enzyme of fatty acid synthesis, could be controlling the chain length of the synthesized fatty acids. No information appears to be available, however, as to whether this control operates in other tissues which usually synthesize a much simpler pattern of fatty acids.

We have recently shown that the particle-free supernatant fraction from homogenates of guinea-pig small intestinal mucosa can synthesize fatty acids from acetate[3]. The present communication describes experiments to investigate whether the pattern of fatty acids synthesized by this fraction can be altered, and if so, whether the

[1] S. SMITH and R. DILS, Biochim. biophys. Acta *116*, 23 (1966).
[2] J. C. BARTLEY, S. ABRAHAM and I. L. CHAIKOFF, Biochim. biophys. Acta *144*, 51 (1967).
[3] M. J. TAME and R. DILS, Biochem. J. *105*, 709 (1967).