When the electrophoresis is carried out in borate buffer, pH 8.6, the mobility of phytohemagglutinins is influenced to a much smaller degree.

The similarity of interactions with carbohydrates of the non-specific hemagglutinins from *P. sativum, L. esculenta, V. cracca* and *C. ensiformis* suggests that all of these substances belong to the same class of phytohemagglutinins with very similar or identical active sites. In addition to the practical usefulness of these reactions for isolation of phytohemagglutinins and quantitative determination of specific carbohydrates[3,11,12], they can no doubt contribute to a better understanding of the mechanism of hemagglutination.

Our work in this direction is being continued.

Fig. 5. Effect of D-glucose concentration on the electrophoretic mobility of pea hemagglutinin. 2% solution of hemagglutinin with different amounts of D-glucose in $0.03 M$ acetate buffer pH 5.0. Other conditions of electrophoresis identical with those given in the legend to Figure 4. D-glucose concentrations: 1, 4.0%; 2, 2.0%; 3, 1.0%; 4, 0.5%; 5, 0.25%.

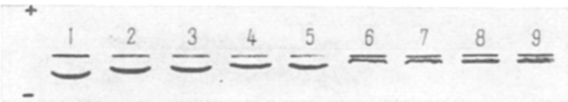

Fig. 6. Effect of various monosaccharides and disaccharides on the electrophoretic mobility of pea hemagglutinin. Conditions of electrophoresis were identical with those given in the legend to Figure 4. 2% solution of phytohemagglutinin in the buffer containing 2% of the following sugars. 1, D-mannose; 2, D-glucose; 3, maltose; 4, D-fructose; 5, sucrose; 6, D-galactose; 7, D-arabinose; 8, D-xylose; 9, cellobiose.

Zusammenfassung. Die in den Samen der Erbse (*Pisum sativum* L.) und der Linse (*Lens esculenta* Moench) enthaltenen Phythämagglutinine reagieren spezifisch auf einige Kohlehydratsubstanzen. Mono- und Oligosaccharide, welche die Agglutinationswirkung dieser Phythämagglutinine hemmen, bewirken auch eine Erhöhung ihrer elektrophoretischen Wanderungsgeschwindigkeit auf Stärkegel. Diese Beschleunigung ist der Hemmwirkung direkt proportionell entgegengesetzt.

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Ethionine and Methionine Biosynthesis in Rat Liver

It is known that the administration of ethionine significantly reduces the transmethylation from methionine for the synthesis of choline[1] and decreases the activity of some enzyme systems involved in choline metabolism (choline oxidase, E.C. 1.1.99.1; sarcosine oxidase, E.C. 1.5.3.1)[2]. Furthermore, ethionine decreases the synthesis of methionine interferring with the metabolic pathway of 1 carbon unit[3].

On the other hand STECKOL et al. have shown that ethionine increases the catabolism of methionine to CO_2, whilst methionine increases the catabolism of ethionine[4].

In order to further clarify the action of ethionine on the methyl transfer in vivo, in this paper the influence of ethionine on the betaine-homocysteine-transmethylase enzymatic system (E.C. 2.1.1.5) has been studied.

Furthermore, since STECKOL[5] has supposed that ethionine could be de-ethylated into homocysteine, comparatively the action of this compound on the same enzyme has been checked.

Experimental procedure. 4 groups of 6 male Wistar rats (250–300 g) were given i.m. L-ethionine. Each of the 4 groups received respectively, 5, 10, 20 and 40 mg of L-ethionine in 2% acqueous solution for 3 days. Another series of rats were given i.m. equimolecular doses of DL-homocysteine.

On the fourth day the animals were bled by decapitation and the betaine-homocysteine-transmethylase activity was determined on the liver by the method of DUBNOFF and BORSOOK[6] and modified by WILLIAMS[7] and ERICSON[8].

The L-ethionine was supplied by the Sigma Chemical Co., the homocysteine by the Nutritional Biochemicals Corp. and the betaine by C. Erba.

Results. From the results reported in Table I it is evident that the transmethylase activity is significantly increased ($p < 0.01$) upon the supply of daily doses of 5, 10 and 20 mg of ethionine. The maximum effect corresponds to the dose of 10 mg; with higher doses the transmethylase activity decreases again toward normal levels.

A similar result has been observed following the administration of homocysteine (Table II). The regression of the enzymatic activity, however, is not so marked as in the case of ethionine.

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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.

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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 γ-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

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