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.

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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

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Comparison of rate of fatty acid synthesis and chain length of synthesized fatty acids

Preparation	Addition	Percentage of incorporated radioactivity in				nmoles acetate incorporated/mg protein/h
		$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	
1	None	0	0	0	0	0
	Citrate (2.5 m*M*)	0	56	43	<1	1.0 ± 0.3
	Citrate (20 m*M*)	0	40 ± 3	59 ± 3	<1	7.8 ± 0.6
	Citrate (40 m*M*)	0	36 ± 2	63 ± 1	<1	5.8 ± 0
2	None	0	66	33	<1	1.0
	Bicarbonate (7.5 m*M*)	0	31	68	<1	1.9 ± 0
	Bicarbonate (20 m*M*)	0	33 ± 3	66 ± 3	<1	5.0 ± 0.3
	Bicarbonate (50 m*M*)	0	28 ± 3	69 ± 2	<3	6.8 ± 0.3
3	None	0	36 ± 1	63 ± 2	<2	5.7 ± 0.1
	0–25% fraction (0.5 mg protein) [a]	0	25 ± 3	75 ± 2	<2	10.5 ± 1.7
	0–25% fraction (1.0 mg protein)	0	25 ± 3	75 ± 2	<2	12.7 ± 0.2
4	None	1	19	79	<1	11.9 ± 0.2
	30–40% fraction (0.5 mg protein) [b]	2	36	62	<2	12.1 ± 0.1
	30–40% fraction (1.1 mg protein)	4	41	56	<1	11.2 ± 0.4
	30–40% fraction (2.1 mg protein)	11	63	26	<5	9.8 ± 0.4

Optimum conditions[3] for fatty acid synthesis were employed except where citrate and bicarbonate concentrations were altered as shown. Incubations were started by the addition of 2.0–2.5 mg protein and in preparation 4, 0.3 mg protein of the 0–25% $(NH_4)_2SO_4$ fraction of the particle-free supernatant was also added to increase the rate of fatty acid synthesis and the proportion of palmitic acid synthesized. $\pm$ represents the mean value from duplicate incubations. In a number of cases, radioactive fatty acids from duplicate incubations were pooled for gas-liquid radiochromatography. [a] The 0–25% $(NH_4)_2SO_4$ fraction and [b] the 30–40% $(NH_4)_2SO_4$ fraction of the particle-free supernatant. The latter was free from acetyl-CoA carboxylase.

pattern is also governed by the rate of synthesis via the concentration of malonyl-CoA.

Methods. The preparation of the particle-free supernatant fraction from homogenates of guinea-pig intestinal mucosa, optimum conditions for fatty acid synthesis from acetate and details of the gas-liquid radiochromatography employed to analyse fatty acids have been previously described[3].

Results and discussion. The Table of results shows that in all cases, the rate of acetate incorporation into fatty acids was lower than that in cell-free extracts of lactating rabbit[1] or rat[2] mammary gland and that fewer types of fatty acids (predominantly myristic and palmitic) were synthesized. Nevertheless, increasing the concentration of citrate (preparation 1) or bicarbonate (preparation 2), and hence the activity of acetyl-CoA carboxylase[3], increased both the rate of acetate incorporation and the proportion of palmitic acid synthesized. Similar results were obtained (preparation 3) when partially purified acetyl-CoA carboxylase (as the 0–25% $(NH_4)_2SO_4$ fraction of the particle-free supernatant[3]) was added to the incubation mixture. This was confirmed using 2 further preparations of the particle-free supernatant.

When fatty acid synthetase (as the 30–40% $(NH_4)_2SO_4$ fraction of the particle-free supernatant[3]) was added (preparation 4), there was only a small change in the rate of acetate incorporation but a very marked reduction in the proportion of palmitic acid synthesized, with increased amounts of myristic and lauric acids formed. Though in other tissues enzymes involved in fatty acid synthesis (such as malonyl-CoA decarboxylase, EC 4.1.1.9 and ATP-citrate lyase, EC 4.1.3.8) appear in this ammonium sulphate fraction[4–6], it is difficult to see how the presence of these enzymes could account for this result. The very marked decrease in chain length of the fatty acids synthesized can be explained, however, by the decreased concentration of malonyl-CoA available to the fatty acid synthetase present. That little change in the rate of fatty acid synthesis occurred shows that the fatty acid synthetase was not rate-limiting.

Conclusion. It has been shown that alteration in the pattern of fatty acids synthesized is not confined to extracts of mammary gland, but can be achieved with extracts of guinea-pig intestinal mucosa. With all these tissues, the proportion of long chain fatty acids synthesized increased with increasing rate of synthesis. The results presented support the suggestion[1,2] that the chain length of the synthesized fatty acids is, at least in part, controlled by the concentration of malonyl-CoA available to the fatty acid synthetase. The mechanism of this control is now being investigated[7].

Résumé. Nous avons montré que la longueur de la chaîne des acides gras synthétisés par les extraits solubles de la muqueuse intestinale du cobaye peut être modifiée. Cette longueur dépend, au moins en partie, de la concentration du malonyl-CoA.

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