komponenten und Pektinsubstanzen pflanzlicher Zellwände, weder durch eigene noch durch mikrobielle Carbohydrasen enzymatisch hydrolysiert (Figur 2), obwohl speziell die Gruppe der Pektinsubstanzen einen Hauptteil des Nahrungsmaterials der Imago ausmacht.

Summary. The activities of β-xylosidase, β-xylanase and inulinase were demonstrated only in the tissues of the larval intestinal canal which also split galactose and arabinose from certain hemicelluloses and ε-galactan. Several other carbohydrases originating from microorganisms were also detected only in the content of the larval alimentary tract.

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S → N Acetyl Migration in Yeast Glyceraldehyde-3-Phosphate Dehydrogenase

In a previous paper we reported that during the hydrolysis of PNPA[1] catalysed by mammalian GAPD a lysine residue is acetylated[2] in addition to the acetylation of cysteine demonstrated by HARRIS et al.[3]. Furthermore we showed[2,4] that N-acetyl enzyme formation is a result of acetyl migration from the intermediary S-acetyl group to the ε-amino group of the lysine residue. The S → N acetyl migration takes place at the active centre and can be prevented by NAD or AMP[4].

Since it could be inferred[5] on the basis of NAD binding and the reactivity of the SH group that the active centre of yeast GAPD is different from that of the muscle enzyme, we examined whether the S → N acetyl migration occurs in the yeast protein, and if so, how this process is affected by the coenzyme and AMP.

S → N acetyl migration was tested by determining the conversion of S-acetyl yeast GAPD, prepared at pH 7.0, into the N-acetyl derivative upon raising the pH to 8.8, as described for the mammalian enzyme[4].

The data show that S-acetyl enzyme is broken down due to the elevation of pH from 7.0 to 8.8: some of the acetyl groups bound originally to the cysteine residue are hydrolysed, while the rest migrate to a neighbouring amino group. ε-N-acetyl lysine could be identified by paper chromatography as described previously[2]. The transformation of S-acetyl GAPD into an N-acetyl derivative is more complete with the yeast than with the muscle enzyme.

In order to study the effect of NAD and AMP on acetyl migration, Tris buffers of pH 8.8 containing the appropriate nucleotide were employed to make the S-acetyl enzyme solutions alkaline. It can be seen in the Table that a considerable amount of N-acetyl enzyme is formed in the presence of the nucleotides. Under the same conditions no N-acetyl GAPD formation was observed with the mammalian enzyme[4].

The S → N acetyl migration exhibited by both muscle and yeast GAPD indicates that the lysine residue is in the environment of the reactive cysteine residue of both enzymes. Another common part of this environment may be the amino acid residues promoting the hydrolysis of the intermediary thiol ester of both enzymes. The hydrolysis of S-acetyl GAPD proceeds at a lower rate with the yeast than with the muscle enzyme and, possibly due to this, the yield in N-acetyl GAPD formation is higher in the former enzyme.

The inhibition of acetyl migration by the nucleotides in the case of muscle GAPD may be interpreted in terms of a simple steric effect or of alterations in the structure of the protein[4]. In the case of the yeast GAPD, however, neither NAD nor AMP prevents the S → N acetyl migration and marked structural changes fail to appear, although under these conditions the enzyme is saturated with coenzyme[6]. We have found that these nucleotides affect only slightly proteolytic digestibility and optical rotation of the yeast protein. NAD-free yeast GAPD exhibits much higher stability than the mammalian enzyme depleted of the bound coenzyme. The yeast protein, however, cannot be protected by the coenzyme or AMP

No. of acetyl groups bound per mole of enzyme at pH 7.0		Nucleotide added at the increase of pH	No. of acetyl groups bound per mole of enzyme at pH 8.8	
S-acetyl	N-acetyl		S-acetyl	N-acetyl
		–	0.12	0.60
1.42	0.15	NAD	0.10	0.47
		AMP	0.15	0.46

56 mg of GAPD was incubated with 2 mg of (acetyl-1-^{14}C) PNPA in 5 ml of 0.05 *M* Tris buffer, pH 7.0, at 5 °C for 2 min. The protein was then freed from excess substrate and reaction products by gel filtering at 5 °C on a Sephadex G-50 column washed with 0.05 *M* Tris buffer, pH 7.0. One fourth of the protein fraction was precipitated with TCA. The remainder was divided into 3 portions and each of them was gel filtered at 5 °C in 15 min through Sephadex G-50 columns washed with 0.05 *M* Tris buffer (pH 8.8), 0.05 *M* Tris buffer (pH 8.8) containing 1.0 mg/ml NAD, and 0.05 *M* Tris buffer (pH 8.8) containing 1.0 mg/ml AMP. The enzyme solutions of pH 8.8 were incubated at room temperature for 30 min, then after precipitating the protein with TCA and washing, the number of total and N-acetyl groups bound to GAPD was determined on the basis of radioactivity of the labelled protein. In order to measure the amount of the N-acetyl groups, the S-acetyl groups had to be removed from the protein. For this purpose the acetyl enzyme was incubated with hydroxylamine solution for 10 min at room temperature. The number of S-acetyl groups was calculated as the difference between the number of total and N-acetyl groups.

[1] Abbreviations: PNPA, *p*-nitrophenyl acetate; GAPD, D-glyceraldehyde-3-phosphate dehydrogenase.

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against spontaneous denaturation as was found in the case of the muscle enzyme[4]. These data, in agreement with previous observations showing the lower affinity of yeast enzyme for NAD[7], suggest that the binding of the coenzyme is different in the two enzymes.

The failure to prevent N-acetyl yeast enzyme formation by the coenzyme indicates that the lysine residue cannot serve as a binding site for NAD. The same conclusion might be drawn from data obtained with the muscle enzyme[4].

Zusammenfassung. Nachweis, dass bei der Hefe-GAPD-katalysierten PNPA-Hydrolyse das als Zwischenprodukt gebildete S-Acetyl-Enzym durch eine intramolekulare Wanderung zu einem N-Acetyl-Derivat führen kann. Im Gegensatz zu dem aus Muskel isolierten Enzym findet im Hefe-Enzym eine Acetylwanderung bzw. eine Acetylierung des gegebenen Lysin statt, ohne wesentliche Beeinflussung durch das Coenzym (NAD).

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Effect of Ethionine Ingestion on Biliary Secretion and Bile Tree Capacity in Rats

The addition of small quantities of ethionine to the diet of experimental animals is found to alter the architecture of the liver. The main histological change described, and one of the first to appear, is proliferation of the ductular system which joins the bile ducts to the canaliculi[1]. Changes in function are also found: the concentration of bile salts being diminished and the flow rate increased[2]. This communication reports the measurement of biliary capacity, distended biliary capacity, flow rate, maximum biliary pressure during obstruction, and dye-concentrating ability of the liver in a group of albino rats given 50 mg ethionine per day for a period of 40 days.

The biliary capacities were measured by a method described previously[3], which consists essentially of an intravenous injection of suitable dye and the measurement of the volume of clear bile pushed out of the tree ahead of the dye-stained bile. The dyes used were bromsulphthalein sodium (BSP) and disodium O-sulphonyl phenyl-di(*p*-sulphonyl benzyl ethylamino phenyl)methanol anhydride (Blue EG), both of which are very rapidly excreted into the bile. Flow rate was measured using a standard drop counter, bile pressure with a Statham transducer, and dye concentrations with an Eel colorimeter.

Both the mean capacity and the mean maximum capacity following obstruction of the bile duct were found to be increased in the ethionine treated animals (Table). In both, the increase measured with BSP was slightly larger than when Blue EG was used, and the increase found following distension was greater than with the undistended tree. The mean biliary flow rate with the ethionine treated animals was 4.5 (SD ± 1.9) ml/h/kg body weight, and with the controls 3.4 (SD ± 0.43) ml/h/kg body weight: an increase of 38%. The mean maximum intrabiliary pressure recorded following obstruction of the bile duct was 12.6 (SD ± 1.8) cm water with ethionine treated rats and 16.5 (SD ± 1.8) cm water with controls: a decrease of 24%. The mean maximum dye concentrations in the ethionine treated rats were 23.2 (SD ± 8.1) mg/100 ml with BSP and 25.2 (SD ± 10.1) mg/100 ml with Blue EG, compared with control values of 39.2 (SD ± 8.7) mg/100 ml and 42 (SD ± 9.8) mg/100 ml respectively, doses of 0.1 mg dye being used in each experiment. The observed increase in bile flow rate is insufficient to account entirely for this fall in concentration.

The residual volume of the original contents remaining in the maximally distended bile tree at different time intervals following the injection of dye was measured in 3 animals. The dead-space volume was substracted from the readings and the results plotted on a log scale (Figure). From this it appears that the removal of bile from the distended tree during obstruction in ethionine treated rats is, as with normal rats[4], exponential with time.

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Increase in biliary capacity in rats fed 50 mg ethionine per day for 40 days

	No. of rats	Mean liver weight (g ± SD)	Mean capacity (µml/g liver ± SD) Undistended		Distended	
			Blue EG	BSP	Blue EG	BSP
Experiment	14	7.3 ± 0.7	4.80 ± 1.2	6.90 ± 0.7	13.20 ± 1.6	15.35 ± 1.4
Control	20	8.4 ± 1.2	3.65 ± 0.5	5.05 ± 0.7	9.85 ± 1.4	10.95 ± 1.0
Increase over control			34%	37%	40%	44%