attached to it[2]. In obesity, this fat often completely replaces large areas of the parenchyma cells of the pancreas.

In the present study, the total lipids of the pancreas show a steep rise with age. Such a rise in the total lipid in relation to age was observed in guinea-pigs by other workers[5,6], this rise being due to triglyceride. The results of the present study with rat pancreas corroborate their findings.

The phospholipid content of the pancreas is highest in very young rats. It decreases with age and is inversely proportional to the level of total lipids. The functions of phospholipids in pancreatic tissue are not clearly understood. Apart from a structural function as a constituent of cell membrane and mitochondria, phospholipids are believed to have a role in protein secretion. HOKIN and HOKIN[7,8] have shown that when protein secretion is stimulated in pancreatic slices, isotopic precursors are increasingly incorporated into phospholipids. During secretion, the rate of labelling of RNA and phospholipids in pancreas is increased without affecting the total lipid content[9].

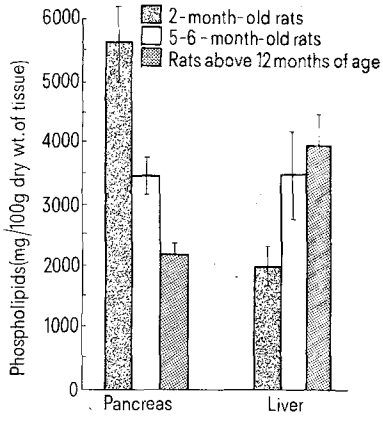

Fig. 5. The phospholipid content in the pancreas.

Both free and total cholesterol of pancreas decrease with age, as observed in the present study. Ester cholesterol, although it rises during growth, diminishes to 50% of its original level in old rats. The diminution of total cholesterol of pancreas due to age parallels that of phospholipid. Further work alone can explain how a diminution of both cholesterol and phopholipid can occur along with increased accumulation of fat with age.

In the liver, the phopholipid is increased while the cholesterol ester is diminished due to age. This is in agreement with the findings of WILLIAMS et al.[10] who made a similar observation with growing rats. However, these workers have not made the study in older rats and the results presented here may therefore be useful for further probe.

Résumé. Le cholestérol et les phospholipides du pancréas des rats diminuent à mesure que l'âge avance, tandisque le contenu total de lipides augmente sensiblement. Dans le foie, les phospholipides augmentent avec l'âge, mais le contenu total de lipide et de cholestérol n'augmentent pas dès que les souris atteignent l'âge de 5 à 6 mois.

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[5] G. E. PALADE and P. SIEKEVITZ, J. biophys. Biochem. Cytol. *2*, 671 (1956).
[6] C. PROTTEY and J. N. HAWTHORNE, Biochem. J. *101*, 191 (1966).
[7] L. E. HOKIN and M. R. HOKIN, Biochem. biophys. Acta *18*, 102 (1955).
[8] M. R. HOKIN and L. E. HOKIN, J. biol. Chem. *203*, 967 (1953).
[9] I. B. BUKHALOV, Biokhimiya *33*, 1255 (1968).
[10] H. H. WILLIAMS, H. GALBRAITH, M. KAUCHER, B. Z. MOYER, A. J. RICHARDS and I. G. MACY, J. biol. Chem. *161*, 475 (1945).
[11] The author is grateful to the Indian Council fo Medical Research, New Delhi for a research grant to carry out this work.

Retarded Enzymatic Degradation of Heterologous Eledoisin Sequences

To protect biologically active peptides against physiological enzymatic degradation, it is suitable to substitute amino acid residues by non-protein constituents, specially in N-terminal position. For example in the case of ACTH, a β-amino acid, or a D-amino acid has been used to get an enhanced activity[1,2].

Recently we were engaged in synthesis and biological testing of hydrazino acids (H_2N–NH–CHR–COOH)[3,4]. They may be interpreted as 'NH-amino acids'. Their introduction in peptides will give 'hydrazino peptides'. As compared with peptides, they contain an additional nitrogen in the peptide backbone.

Replacement of the α-carbon in amino acid residues by nitrogen leads to α-azapeptides, the second type of heteropeptides used in these investigations.

····–CO–NH–CHR–CO–NH–····	peptide
····–CO–NH–NH–CHR–CO–NH–····	hydrazino peptide
····–CO–NH–NR– –CO–NH–····	α-aza-peptide

Synthesis of hydrazino or aza analogues of 5-asparagine-eledoisin peptides[5,6] mentioned here and their biological activity in isolated organs and on blood pressure in fowl, guinea-pig, and rat were published earlier[7].

Eledoisin:

pGlu–Pro–Ser–Lys–Asp–Ala–Phe–Ile–Gly–Leu–Met–NH_2
1 2 3 4 5 6 7 8 9 10 11

(positions of the heteroconstituents are underlined).

[1] K. INOUYE, A. TAMAKA and H. OTSUKA, Bull chem. Soc. Japan *43*, 1163 (1970).
[2] W. DOEPFNER, Experientia *22*, 527 (1966).
[3] P. OEHME, H. REX, A. WERGIN and E. ACKERMANN, Acta biol. med. germ. *21*, 635 (1968).
[4] H. NIEDRICH and W. KNOBLOCH, J. prakt. Chem. *17*, 263, 273 (1962).
[5] H. NIEDRICH and CH. OEHME, J. prakt. Chem. *314*, 759 (1972).
[6] R. GRUPE and H. NIEDRICH, J. prakt. Chem. *312*, 1087 (1970). — Chem. Ber. *100*, 3273, 3283 (1967).
[7] P. OEHME, J. BERGMANN, H. NIEDRICH, F. JUNG and G. MENZEL, Acta biol. med. germ. *25*, 613 (1970); *28*, 109, 121 (1972).

In this paper we wish to report on the susceptibility of eledoisin heterosequences towards hydrolysis by organ homogenates and aminopeptidases.

Materials and methods. The organ homogenates were prepared by a method published by NOBILI[8]. Aminopeptidase M (AP-M) was purchased from Röhm and Haas Co., Darmstadt. Pure leucine aminopeptidase (LAP) from bovine eye lenses was kindly supplied to us by Prof. HANSON, Halle (GDR). Peptide substrates were incubated with organ homogenates at 37 °C. The rate of cleavage was estimated by measuring the contraction of residual peptide in isolated guinea-pig ileum after dilution to the suitable concentration range of the organ bath. Incubation sample contains 0.3 ml peptide solution, 2.55 ml phosphate buffer, pH 7.4, and 0.15 ml homogenate from 40 mg moist organ. The peptide concentration was 2.4×10^{-7} *M*.

In the experiments with aminopeptidases (for results see Table) 350 mU AP-M or 1.5 mg LAP were used per µmol peptide for enzymatic hydrolysis of eledoisin heterosequences. Conditions were: 20 h, 37 °C, pH 8.9 for LAP and 7.7 for AP-M. Incubation was followed by determination of the amino acid released, using an automatic amino acid analyser.

Hydrolysis of heteropeptides by aminopeptidases

Peptide	% Recovery for			
	Lys	Ala	Ile	
Lys-Asn-Ala-Phe-Ile-Gly-Leu-Met-NH_2	76	70	73	LAP
Lys-Azasn-Ala – – – – – – – – – – I	78	1	1	LAP
Lys-N(Ac)-Gly-Ala – – – – – – – – – – I	7	2	5	AP-M
Ala-NHPhe(Ac)-Ile – – – – – – – – – I	—	73	3	AP-M
Ala-N(Ac)-Phe-Ile – – – – – – – – – – I	—	2	2	AP-M

Azasn = –NH–N(CH_2–$CONH_2$)CO–, N(Ac)—Gly = N^{α}-acetyl hydrazinoacetyl (–NH–N^{α}(Ac)–CH_2–CO), NHPhe = α-hydrazino-β-phenylpropionic acid.

Results and discussion. The following guinea-pig organs were used: liver, kidney, ileum, skeletal muscle, lungs, spleen, uterus, tube, seminal vesicle. While complete inactivation of 5-Asn-eledoisin-4-11 and analogues modified in position 7 and 9 occurs with most of the organ homogenates within 15–30 min, no loss in activity of 5-Azasn-eledoisin-4-11 during the same period of time was observed. Only liver homogenate shows a low rate of degradation with 50% cleavage in about 30 min.

Unmodified eledoisin sequences are cleaved completely by aminopeptidases; but in heterosequences with hydrazino carbonic acids or aza amino acids, only the amino acid residue just before the heteroconstituent is removed. In acetylated hydrazino peptides, the enzyme only eliminates a trace of the amino acid preceding the heteroconstituent. Such modification in cleavibility may certainly be attributed to deviation from the natural peptide structure.

In hydrazino peptides, the alterations are a chain elongation of about 1.2 Å and a torsion of about 60° for the side chain of the hydrazino acid or for the subsequent peptide chain, as judged by molecular models. Azapeptides contain a relatively rigid semicarbazide structure, caused by their urea-like mesomerism[5]. The consequence would be a considerable steric change in the azapeptide molecule. This assumption is supported by a significant alteration in circular dichroism spectra (1 positive maximum at 210 nm) and by the fact that also chymotrypsin and pronase show a retarded inactivation of 5-Azasn-eledoisin-4-11.

Zusammenfassung. Analoge des Eledoisin-Oktapeptides 4-11, die Hydrazid-Komponenten enthalten, sind durch Aminopeptidasen nur bis zur Heterobindung abbaubar. Sie werden wesentlich langsamer als die native Sequenz bzw. nicht durch Organhomogenate inaktiviert, wenn die Substitution im N-terminalen Bereich erfolgt. Mögliche sterische Veränderungen des Peptids durch eingebaute Fremdbausteine sind diskutiert.

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6 February 1973.

[8] M. B. NOBILI: Archs inter. Pharmacodyn. *158*, 187 (1965).

Phosphatases IX[1]. Differences in Sialic Acid Content of Rat Liver Alkaline Phosphatase Isoenzymes

The heterogeneity of alkaline phosphatase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1) within a single tissue, as demonstrated by gel electrophoresis of tissue extracts, has frequently been noted[2–4]. For rat liver 2 main isoenzymes have been found[5–7]. In our previous experiments, different response of the 2 isoenzymes to bile duct ligation was reported[5]. The present communication deals with the different response of the 2 isoenzymes to neuramidase treatment.

Material and methods. Adult male Wistar rats (approx. 200 g body wt.), fed a standard food diet and fresh water ad libitum, were killed by decapitation. The liver was perfused with ice-cold 0.25 *M* sucrose, rapidly removed, weighed and homogenized in cold 0.25 *M* sucrose with a Potter-Elvehjem homogenizer fitted with a Teflon-pestle. The nuclear and mitochondrial fractions were prepared according to APPELMANS et al.[8]. Both fractions were washed 3 times. Alkaline phosphatase was extracted from the homogenate and from the subcellular fractions[9]. *n*-Butanol was added to a final concentration of 20%, the mixture was warmed up to 37 °C and stirred for 15 min. The aqueous phase was obtained after centrifugation at 20,000 x *g* (4 °C) for 30 min, dialyzed overnight against 0.05 *M* sodium acetate and used for the experiments.

The preparations were incubated with neuraminidase at 37 °C overnight. 1 mg of neuraminidase from *Clostridium perfringens* (Sigma) was dissolved in 0.1 ml of 0.1 *M* sodium acetate, 0.15 *M* NaCl, 0.01 *M* $MgCl_2$ at pH 5.5.