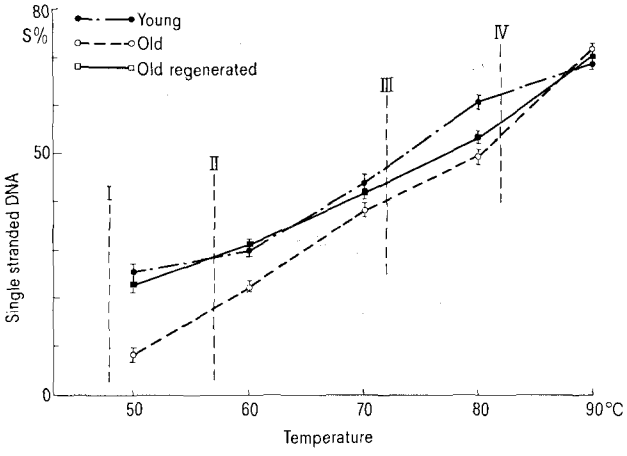
the protein-free DNA fraction of the nucleus, amounting to 25% in the young animals and only to 8% in the old ones. It is remarkable that the regenerated old liver reached almost the young value. The percentage of protein free DNA in young livers is of the same order as observed in other tissues using also quite different techniques by others (18–25%)[13,16–18]. One can assume, therefore, that, for the normal function of interphase cell nucleus, about this proportion of DNA should be in a protein-free state. In the old animals this value decreased to a high extent which must have serious functional consequences. The percent of single stranded DNA obtained at 60 °C is to be considered as the sum of free DNA and DNA stabilized by non-histone proteins, i.e. in the young animals only about 4% of DNA is stabilized by acidic proteins; in old rats this value increased to about 13%, whereas in the regenerated old liver it was between these values. Following a similar logic, one can see that the more basic part of histones (melting point at 82 °C)[16] is binding a higher portion of DNA in the old liver than in the young one, and again the regenerated old liver lies between these values.



Thermal denaturation curves of nuclear DNA in rat liver. S = single stranded DNA in % of DNA-phosphate. Dotted lines and roman numbers indicate the sites of peaks of derivative melting profile[15]. The plots are taken from the Table. (Means ± SEM). Significance values: A) Young-old comparison: $p < 0.1\%$ at each temperature, except 90 °C where N.S. B) Young-old regenerated comparison: N.S. except 80 °C where $p < 0.1\%$. C) Old-old regenerated comparison: $p < 0.1\%$ at 50 and 60 °C; $p < 2\%$ at 80 °C; N.S. at 70 and 90 °C.

We believe that our results allow us to conclude a certain reversibility of the age-dependent condensation of chromatin by mitoses, supporting the idea that true ageing phenomena are present first of all in the post-mitotic cells[19].

From a methodical point of view, our results show that the in situ thermal denaturation with parallel measurement of the DNA-loss, introduced in our laboratory[13], is a valid method for studying the physico-chemical properties of chromatin.

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Exocrine Pancreatic Enzymes in Cycloheximide Treated Rats

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Summary. Cycloheximide, even in a dose of 0.25 mg/kg administered s.c. to rats stimulated by pancreozymin and secretin, inhibited lipase activity in pancreatic juice. Lipase activity in serum of control animals was inhibited by cycloheximide. The secretion of trypsin and chymotrypsin was also decreased.

Cycloheximide, an antibiotic isolated from *Streptomyces griseus*[1], inhibits synthesis of proteins in yeast and mammalian systems by interfering with both the initiation and elongation of polypeptide chains on polyribosomes[2]. The drug has been used extensively to study the relationship between a number of biological processes and protein synthesis[3–9]. Moreover, cycloheximide has been used clinically[10] recently to produce defervescence in chronically febrile patients with Hodgkin's disease[11]. In our laboratories we extend the earlier observation[12] that cycloheximide affects gastric secretion. Especially gastric acidity was almost completely abolished by a single dose of the drug[13]. In this report evidence is presented that exocrine secretion of some enzymes in the pancreas and in the serum of rats are also inhibited following the administration of this drug.

Materials and methods. Experiments were performed on 100 male Wistar rats (240–250 g) kept on a standard diet. The first group of animals was treated s.c. with one dose, or with the same dose of cycloheximide for 3 consecutive days. The animals were killed on the 2nd or the 4th day,

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Effect of cycloheximide on stimulated pancreatic secretion in rats

Conditions	Trypsin (mg/g protein ± SE)	%	Chymotrypsin (mg/g protein ± SE)	%	Lipase (mg/100 ml)	%
Control	0.331 ± 0.120[a]	(100)	0.364 ± 0.140[a]	(100)	3.33 ± 0.62	(100)
Pz	1.101 ± 0.380[a]	(332)	2.760 ± 0.908[a]	(730)	4.38 ± 0.97[b]	(131)
Pz + CHX	0.930 ± 0.433	(250)	2.130 ± 0.587	(585)	2.73 ± 0.13[b]	(82)
Control	0.423 ± 0.173	(100)	0.384 ± 0.141	(100)	2.18 ± 0.52[a]	(100)
St	0.475 ± 0.165	(112)	0.410 ± 0.149	(107)	4.79 ± 0.95[a,b]	(219)
St + CHX	0.394 ± 0.109	(92)	0.363 ± 0.021	(94)	2.45 ± 0.19[b]	(112)

Groups of 10 male rats (220 g) kept under standard condition received pancreozymin (Pz, 4 units per 200 g, i.v.) at 09.00 h (e.g. 1 h after operation). 20 min later the same amount of secretin (St) was given and pancreatic secretion was followed 1 more hour. Cycloheximide (CHX, 0.25 mg per kg, s.c.) or saline were given 20 min before starting the experiment. [a] $p < 0.05$; [b] $p < 0.05$.

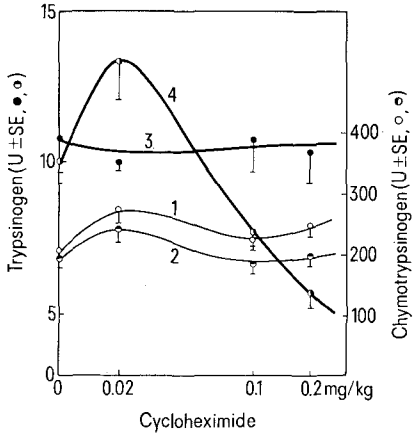

Fig. 1. Activity of proteolytic enzymes in the homogenate of pancreas of rats treated with 1 dose (1,3) and 3 doses of cycloheximide given i.p. for 3 consecutive days (2,4) to groups of 5 male rats (220 g). 1,2-Chymotrypsinogen expressed as mg of crystalline enzyme per 1 g of protein; 3,4-trypsinogen expressed as crystalline enzyme per 1 g of protein.

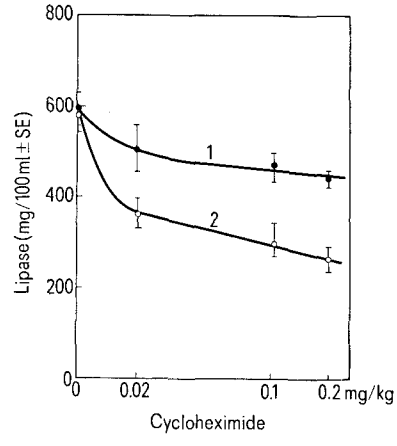

Fig. 2. Decrease in lipase activity in the serum of rats treated with one (1) dose and 3 doses of cycloheximide given i.p. for 3 consecutive days (2) to groups of 5–6 male rats (220 g). Lipase activity is expressed as mg of reaction product per 100 ml of serum ± SE ($p < 0.01$).

and pancreatic enzymes in homogenated pancreas and in serum were measured. In the 2nd group of animals, we studied the effect of cycloheximide on pancreatic secretion stimulated with pancreozymin and secretin (Boots Comp.). Polyethylene cannules were placed in the duodenum and the pylorus was ligated in mild ether narcosis in all experiments at the same time (08.00 h).

The activity of trypsin and chymotrypsin was measured in the pancreas homogenized in a medium containing 0.5% NaCl and 0.05% $CaCl_2$ (pH 5.2). Homogenization was performed in cooled glass homogenizer with 10 vol. of the media (w/v). The activity of proteinases was measured photometrically after their activation by enterokinase (Organon) or by trypsin (Spofa) using synthetic substrates N-tosyl-L-arginine-*p*-nitroanilide for trypsin[14] and N-succinyl-L-phenylalanine-*p*-nitroanilide (Boehringer) for chymotrypsin. Lipolytic activity was determined photometrically using phenyllaurate according to method of RADERECHT[15]. Amylase was determined by the method of TELLER[16]. The protein content was estimated according to LOWRY et al.[17]. The activity of enzymes was expressed in mg of trypsin or chymotrypsin per 1 g of the tissue proteins or in mg of the reaction product (lipase – phenol) per 100 ml of serum.

Results and discussion. Cycloheximide was given at doses varying from 0.02 to 0.2 mg/kg body weight without any stimulation of pancreatic secretion. There are differences, depending whether the drug was given in 1 or for 3 consecutive days (Figure 1). The increasing doses of cycloheximide resulted in the second case in the inhibition of trypsinogen level in the pancreatic homogenates. Also the level of serum lipase (Figure 2) was significantly depressed following a single dose of cycloheximide. However, the changes in chymotrypsinogen under similar conditions were not significant (Figure 1).

When the pancreatic secretion was stimulated with pancreozymin and secretin cycloheximide at a dose of 0.025 mg per kg also lipase activity was inhibited (Table). The inhibition was statistically significant. The secretion of trypsin and chymotrypsin was also decreased but the

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changes were not significant. There were no differences in amylase activity between control and experimental animals.

Secretion of digestive enzymes from pancreas operates via the pathway which is Ca^{2+}-dependent and involves cholinergic and peptide hormone receptors[18,19]. In this study the effect of cycloheximide on the activity of lipase, amylase, trypsin and chymotrypsin present in rat pancreas, as well as circulating in the serum, was measured. To understand the cycloheximide-induced inhibition of exocrine pancreatic enzymes, one has to take into consideration the inhibitory effect of the drug on gastric secretion[13]. The activity of pancreatic enzymes depends on and is modified by end products of digestion appearing in the blood[20–23]. In this way the impaired digestion and the delay of gastric emptying caused by cycloheximide, simulating to some degree starvation of the animals, can indirectly affect the level of exocrine pancreatic enzymes.

Following cycloheximide administration, direct inhibition of enzyme synthesis in acinar cells can also be expected and the interference of the drug with receptors necessary for enzyme secretion cannot be excluded. Decreased activity of lipase and trypsin following cycloheximide administration and significantly decreased pathological changes in histological sections evaluated in experimental pancreatitis following drug treatment (unpublished) present potential opportunities for the use of cycloheximide in gastroenterology.

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[^{3}H]-Norcocaine and [^{3}H]-Pseudococaine: Effect of N-Demethylation and C_2-Epimerization of Cocaine on its Pharmacokinetics in the Rat[1]

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Summary. After i.v. injections of cocaine, norcocaine, pseudococaine to the rat, the $T_{1/2}$ in brain were 0.4, 0.6, 0.2 h respectively and in plasma 0.4, 0.5, 0.2 h respectively. Benzoylnorecgonine and norecgonine were the metabolites of norcocaine in brain. Pseudonorcocaine, pseudobenzoylnorecgonine, pseudobenzoylecgonine and pseudoecgonine were the metabolites of pseudococaine in rat brain. Benzoylnorecgonine and pseudobenzoylecgonine had potent stimulant activity intracisternally in the rats.

Norcocaine (Figure 1) has been reported to be an important metabolite of cocaine in the rat[2,3] dog[4] and monkey[5]. Like cocaine, it is a powerful central nervous system stimulant[6,7] of short duration and it contributes possibly in part to the pharmacological effects of cocaine. Pseudococaine (dextro isomer and C_2-epimer of cocaine) has the C_2-methyl ester group in equatorial configuration and *trans* to the nitrogen and C_3-benzoyloxy side chain[8] (Figure 1). Its toxicity and local anesthetic activity is several-fold higher than cocaine[9–11]. Both isomers have convulsant and paralysant properties[10–14], but pseudococaine is several times less potent than cocaine as an inhibitor of norepinephrine uptake in ventricles and vas deferens slices[13,15]. It has been reported to be more extensively degraded than cocaine[16,17] in cats. No information exists on the dispositional and metabolic profile of norcocaine and pseudococaine in the central nervous system. This study was undertaken to obtain information on these parameters.

Materials and methods. Norcocaine was prepared by a procedure previously described[18]. Pseudococaine was prepared by conversion of cocaine to pseudo ecgonine methyl ester[8] and subsequent benzoylation with benzoylchloride in benzene using standard procedures. Samples (50 mg) of these non-labelled compounds were tritiated (New England Nuclear, Boston, Mass.) by acid-catalyzed exchange tritium labelling using 0.3 ml acetic acid, 25 mg platinum catalyst and 10 curies of tritiated water. The crude products were exhaustively purified in our laboratory by repeated solvent extractions and silica gel column chromatography (eluant, benzene-methanol

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