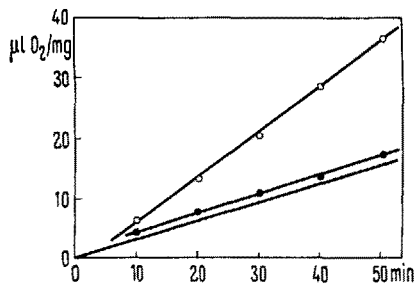

Fig. 1. Oxydation of glucose and hexadecane by protoplasts of *Candida lipolytica*. —○— glucose (0.4 mg/3.0 ml); —●— hexadecane (0.2 ml/3.0 ml); — endo.

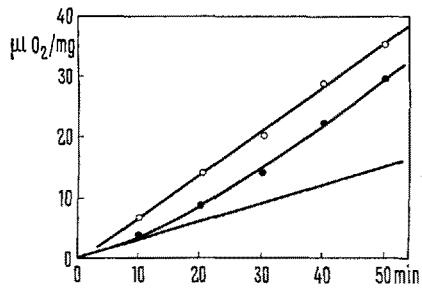

Fig. 2. Oxydation of glucose and hexadecane by intact cells of *C. lipolytica*. —○— glucose (0.4 mg/3.0 ml); —●— hexadecane (0.2 ml/3.0 ml); — endo.

decane was measured in the Warburg apparatus. The results of the experiments with intact cells and protoplasts are shown in Figures 1 and 2.

While the enzymatic glucose oxidation with the protoplasts of *C. lipolytica* towards the intact cells showed no changes, complete loss of oxidation activity on hydrocarbons was observed. This phenomenon was not due to contingent washing out of enzymes into the environment, as even the unwashed suspension of protoplasts in initial digestive environment showed no oxidation activity on hexadecane. Also the other substances such as cystein, 2-mercaptoethanol or digestive juice of *H. pomatia* inactivated by heat, did not inhibit the oxidation of hydrocarbons by intact cells.

We presume that the protoplasts lost their ability to oxidize the hydrocarbons either by change in the surface of the cytoplasmic membrane and destruction of oxidative enzymes bound on it, or by loss of protoplast ability to transport the hydrocarbons into the cell.

Zusammenfassung. Aus den Zellen der Hefe *Candida lipolytica* wurden Protoplasten gewonnen, die die Oxydationsfähigkeit gegenüber Hexadekan verloren haben, während diejenige gegenüber Glukose erhalten geblieben ist. Der Verlust der Fähigkeit, den Kohlenwasserstoff zu oxydieren, wird entweder durch eine Veränderung der Oberfläche der zytoplasmatischen Membran und Destruktion des an sie gebundenen Oxydationsenzyms erklärt oder dadurch, dass die Protoplasten die Fähigkeit verloren haben, Kohlenwasserstoffe in die Zelle zu transportieren.

O. VOLFOVÁ, V. MUNK and M. DOSTÁLEK

Department of Technical Microbiology, Institute of Microbiology, Czechoslovak Academy of Science, Praha 4 (Czechoslovakia), 19 May 1967.

Influence of Pyridinolcarbamate on Hepatic Cholesterogenesis in Rats

Pyridinolcarbamate (2,6-*bis*(hydroxymethyl) pyridine di-N-methylcarbamate) has been reported by SHIMAMOTO to inhibit experimental atherosclerosis in rabbits and to ameliorate various forms of atherosclerotic heart disease in man[1,2]. There are, currently, no published data concerning the influence of this compound on cholesterol synthesis, absorption or degradation. This communication describes the influence of dietary pyridinolcarbamate (0.3%) upon the synthesis of cholesterol by rat liver slices.

One group of male Wistar rats were maintained for 3 weeks on a diet consisting of mixed cereal 70%, wheat germ 7%, skim milk powder 21% and vitamin mix 2%. Another group was fed this diet augmented with 0.3% pyridinolcarbamate. The diet contained 20% protein, 11% fat and 62% carbohydrate. It is readily accepted by the rats and has proved to be an excellent vehicle for administration of test compounds[3,4].

After 3 weeks the rats were decapitated, and the livers excised and washed in chilled phosphate buffer. Liver slices (0.5 g) were incubated for 3 h under 100% oxygen in 5 ml of phosphate buffer (pH 7) containing 0.006 M $MgCl_2$, 0.03 M nicotinamide and either 1 μc of sodium acetate-1-^{14}C or 0.5 μc of mevalonic acid-2-^{14}C. The reaction was stopped by the addition of hot 15% alcoholic KOH; the cholesterol was extracted from the saponification mixture and isolated as the digitonide. The radioactivity of the cholesterol digitonide was then assayed by liquid scintillation spectrometry[5]. Another portion of the liver was saponified in 15% alcoholic KOH and the cholesterol then extracted. The total serum and liver cholesterol levels were determined by the method of MANN[6].

The results of 2 experiments are summarized in the Table. The serum and liver cholesterol levels of the rats that were fed 0.3% of pyridinolcarbamate did not differ

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Influence of pyridinolcarbamate (0.3%) on serum and liver cholesterol and cholesterol synthesis in rats (compound fed 3 weeks)

Group[a]	No.	Weight gain (g)	Liver weight (g)	Cholesterol levels		Cholesterol biosynthesis[b]	
				Liver g/100 g	Serum mg/100 ml	Acetate-1-^{14}C (1 μc)	Mevalonate-2-^{14}C (0.5 μc)
Experiment 1							
P	6	42	8.7	370 ± 31[c]	38.9 ± 1.8	0.37 ± 0.02	1.06 ± 0.13
C	6	52	10.0	384 ± 21	33.2 ± 4.3	0.93 ± 0.24	1.14 ± 0.12
Experiment 2							
P	5	106	11.8	364 ± 30	43.2 ± 9.8	0.82 ± 0.20	–
C	5	112	12.4	299 ± 8	47.3 ± 3.6	3.40 ± 0.69	–

[a] P, pyridinolcarbamate; C, control. [b] % conversion. [c] Standard error.

from those of the controls. SHIMAMOTO et al.[2] reported that administration of 10 mg/kg of the test compound to rabbits maintained on 1% cholesterol for 12 weeks did not affect serum cholesterol levels but reduced the cholesterol content of the aorta. The controls and drug-fed rats exhibited no differences in weight gain; the animals in the first experiment weighed 174 ± 3 g at the beginning of the experiment, while those in the second experiment weighed 206 ± 3 g when feeding was begun. Liver weights of the pyridinolcarbamate-fed rats were slightly lower than those of the controls, but the differences were not significant. The liver weight, as % of body weight, was lower in the pyridinolcarbamate-treated rats than in the controls (3.97% vs 4.48% and 3.75% vs 3.93% in experiments 1 and 2, respectively).

Incorporation of acetate-1-^{14}C into cholesterol by rat liver slices was significantly reduced in the pyridinolcarbamate-fed rats ($0.05 > p > 0.02$ in experiment 1 and $0.01 > p > 0.001$ in experiment 2). There was no effect observed in the experiment involving cholesterol biosynthesis from mevalonate-2-^{14}C.

There is no evidence that the effects of pyridinolcarbamate upon human and experimental atherosclerosis[1,2] are mediated through its inhibition of cholesterol synthesis. Nicotinic acid inhibits experimental atherosclerosis in rabbits[7] and has been reported to inhibit cholesterogenesis by some workers[8,9] (but not others[10,11]). However, its hypocholesteremic and anti-atherogenic effect is generally conceded to be due to the inhibition of free fatty acid mobilization[12,13]. The elucidation of the mechanism of action of pyridinolcarbamate must await further investigation[14].

Zusammenfassung. Männliche Wistar-Ratten wurden während 3 Wochen mit 0,3% Pyridinolcarbamat gefüttert, der Cholesterinspiegel in Leber und Serum bestimmt und ausserdem der Einbau von Na-Acetat-1-^{14}C und Mevalonsäure-2-^{14}C im Cholesterin von Leberschnitten gemessen. Der Cholesterinspiegel in Serum und Leber wird durch Pyridinolcarbamat nicht beeinflusst. Der Einbau von Acetat-1-^{14}C in Cholesterin wurde durch Pyridinolcarbamat gehemmt, während die Conversion von Mevalonat-2-^{14}C unbeeinflusst blieb.

D. KRITCHEVSKY and SHIRLEY A. TEPPER

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Partial Characterization of Human Pancreatic Carboxypeptidase A

Studies of the chemical and enzymic characterization of bovine[1,2], porcine[3,4] and canine[5] pancreatic carboxypeptidases A (EC 3.4.2.1) have been achieved during the last several years by many workers. However, human pancreatic carboxypeptidase A has never been isolated and characterized to our knowledge. The purpose of this present report is the partial characterization of human pancreatic carboxypeptidase A and the comparison of some of its enzymic properties with those of the bovine and porcine enzymes.

To obtain a purified carboxypeptidase A preparation, 16.9 g of the acetone powder which was prepared from 7 pancreas glands (160 g) by the method of KELLER et al.[6] were extracted with 350 ml of water (dry weight, 10.7 g; $C_1 = 0.022$[7]). The following fractionation with ammonium sulphate was performed with 39% saturation at pH 7.4 (precipitates, 880 mg; $C_1 = 0.049$). For further purification, the precipitates were submitted to chromatography on DEAE-cellulose (linear buffer gradient: 0.005–0.3 *M*