Die hohen spezifischen Aktivitäten des Threonin sind bemerkenswert. Umgerechnet ergibt sich, dass rund 20% des Threoningehaltes in Kollagen vom Prolin stammen. Weitere Untersuchungen sollen Auskunft über diesen – unseres Wissens bisher unbekannten – Weg im Intermediärstoffwechsel des Prolins geben.

Summary. Beside hydroxyproline collagen contains quantities of glutamine, threonine, serine, alanine and glycine, derived from proline. Remarkably large quantities of threonine have this origine, which suggests that conversions occur in the fibroblast.

A. RUIZ-TORRES und I. KÜRTEN

Stoffwechselabteilung an der Medizinischen Klinik und Poliklinik der FU Berlin im Klinikum Westend, Spandauer Damm 130, D-1 Berlin 19 (Deutschland), 28. Februar 1973.

Hepatic Drug Metabolizing Activity in Lead-Poisoned Rats[1]

Lead poisoning of young children in slum areas of major cities[2–4] has produced a reawekened interest in the study of lead-induced biochemical effects. An extensive literature[5] reports alterations of a series of metabolic processes observed after acute or chronic exposure to inorganic lead.

In particular, the mixed function oxidase system of liver endoplasmic reticulum is impaired in acute lead poisoning[6]. The activity of this enzyme system, responsible for the metabolism of a large number of xenobiotics, is closely related to the availability of the microsomal hemoprotein cytochrome P-450[7]. Lead-induced inhibition of δ-aminolevulinic acid dehydrase[8–10] may result in inhibition of heme synthesis and therefore in reduced levels of cytochrome P-450 with consequent impairment of the mixed function oxidase system.

The purpose of the present investigation was to provide further evidence for lead-induced modifications of hepatic drug metabolism and to demonstrate a relationship between impairment of the mixed function oxidase system and inhibition of δ-aminolevulinic acid dehydrase activity.

Materials and methods. Male albino rats (Sprague-Dawley derived, CD strain), with an average body weight of 160 g, were used. The animals were allowed at least 4 days to acclimatize to our animal quarters and were treated i.v. with lead nitrate. Control groups received isotonic sodium chloride. The rats were killed by decapitation between 09.00 and 10.00 h; the livers were quickly removed, chilled in ice-cold 0.1 *M Tris*-HCl pH 7.5, perfused with the same buffer to remove blood, and weighed. All remaining procedures were carried out at 0–4 °C unless otherwise indicated. The livers were minced with scissors in 0.1 *M Tris*-HCl pH 7.5 and 25% (w/v) homogenates prepared with a Potter-Elvehjem homogenizer having a plastic pestle. Homogenates were centrifuged at 12,000 g_{av} for 20 min. The pellet was discarded and the post-mitochondrial supernatant used for enzymic and chemical assays. Cytochrome P-450 and enzyme activities were measured within 4 h after the animals had been killed.

N-Demethylation of aminopyrine was determined by measuring the formation of the intermediate metabolite 4-aminoantipyrine[11] and that of formaldehyde[12]. *O*-Demethylation of *p*-nitroanisole was assayed by the method of NETTER and SEIDEL[13], as modified by SCHOENE et al.[12] for kinetic determinations. Aromatic ring hydroxylation was tested, measuring the formation of *p*-acetaminophenol from acetanilide[14] and that of *p*-aminophenol from aniline[14]. Incubations were carried out in a Dubnoff shaking water bath (125 oscillations/min) at 37 °C under air. A glass marble was added to each incubation vessel to facilitate mixing of the contents[15].

δ-Aminolevulinic acid dehydrase activity was measured with the method of BONSIGNORE et al.[16]. Cytochrome P-450 was determined according to the procedure recently described for human liver homogenates[12]. Difference spectra were recorded with a Perkin-Elmer 350 double beam spectrophotometer equipped with an external device for scale expansion. A Gilford 2400 spectrophotometer with wavelength programmer and reference compensator was routinely used for alternate measurements at 450 and 490 nm. Protein content was estimated by the method of LOWRY et al.[17]; crystalline bovine serum albumin served as the reference standard. Lead in liver homogenates and post-mitochondrial supernatants was determined with the dithizone method after digestion with nitric-perchloric acid[18] or by γ-spectrometry in some experiments carried out with lead nitrate labelled with ^{203}Pb (kindly provided by the Institute of Physics, University of Milan). Pentobarbital sleeping time was determined after i.v. injection of 40 mg/kg body wt. by measuring the duration of loss of the righting reflex. Statistical analysis was performed with the Student *t*-test, using a top-desk computer Olivetti 101.

Results and discussion. A dose dependent increase in liver weight was observed in lead-poisoned rats. On the

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Lead, cytochrome P-450 and drug metabolizing activities in rat liver after a single dose of lead nitrate

Animal group	Lead content (μg/g liver)		Cytochrome P-450	Aminopyrine N-demethylase		*p*-Nitroanisole *O*-demethylase	Acetanilide hydroxylase	Aniline hydroxylase
	Whole liver homogenate	Post-mitoch. supernatant	(pmol/mg protein)	Aminoantipyrine (pmol/min/mg protein)	Formaldehyde (pmol/min/mg protein)	*p*-Nitrophenol (pmol/min/mg protein)	*p*-Acetaminophenol (pmol/min/mg protein)	*p*-Aminophenol (pmol/min/mg protein)
Control (40)	< 0.2	–	218 ± 3	112 ± 2	3616 ± 42	607 ± 7	452 ± 5	526 ± 8
100 μmoles/kg								
1 day (8)	55.8 ± 7.3	24.9 ± 2.3	102 ± 5 −53%	39 ± 5 −65%	1808 ± 70 −50%	243 ± 11 −60%	191 ± 10 −58%	257 ± 11 −52%
2 day (4)	28.4 ± 8.0	14.1 ± 2.4	94 ± 11 −57%	29 ± 7 −74%	1519 ± 102 −58%	201 ± 24 −67%	167 ± 19 −63%	225 ± 18 −58%
3 day (4)	14.0 ± 1.5	8.9 ± 0.6	153 ± 15 −30%	49 ± 6 −56%	2206 ± 195 −39%	316 ± 36 −48%	271 ± 23 −40%	338 ± 29 −37%
5 day (4)	5.3 ± 0.6	2.8 ± 0.2	182 ± 18 −16%	86 ± 10 −23%	2857 ± 236 −21%	504 ± 54 −17%	375 ± 31 −17%	461 ± 42 −14%
7 day (4)	3.1 ± 0.2	1.8 ± 0.1	211 ± 20 − 3%	101 ± 12 −10%	3290 ± 232 − 9%	570 ± 51 − 6%	434 ± 36 − 4%	542 ± 47 + 3%
50 μmoles/kg 1 day (8)	29.4 ± 1.3	15.2 ± 0.7	126 ± 6 −43%	53 ± 3 −53%	1916 ± 83 −47%	297 ± 13 −51%	280 ± 15 −38%	306 ± 13 −43%
10 μmoles/kg 1 day (8)	6.5 ± 0.3	3.2 ± 0.1	153 ± 7 −30%	81 ± 4 −28%	2495 ± 97 −31%	449 ± 18 −26%	339 ± 12 −25%	423 ± 16 −21%

All values are means ± S.E.; number of animals given in parentheses.

first day after lead nitrate administration, relative liver weight (g liver/100 g body wt.) was significantly higher than in control animals. Protein concentration in the post-mitochondrial supernatant was slightly reduced. The increase in liver weight accompanied by a decrease of protein concentration is in good agreement with typical lead-induced pathologic alterations, characterized by mitochondrial and lysosomal damages resulting in inhibition of oxidative phosphorylation and release of lysosomal enzymes[19].

The amount of lead present in the liver after i.v. administration of lead nitrate (10 to 100 μmoles/kg) was dose-proportional and decreased with a kinetic represented by the sum of 2 exponential terms. The post-mitochondrial supernatant, consisting of microsomes and cytoplasmic soluble fraction of hepatic cell, contained approximately 50% of the lead found in whole-liver homogenate. Hepatic cytochrome P-450 and drug metabolizing activities were significantly decreased in lead-poisoned animals. Intensity and duration of these changes were dose-dependent. When lead nitrate had been administered at the highest dose, both cytochrome P-450 and enzyme activities remained reduced for several days. Normal levels were regained when hepatic lead concentration fell below 3 μg/g liver. In vivo experiments, based on the duration of pentobarbital sleeping time, provided further evidence for inhibition of drug metabolism in lead-poisoned rats. Narcosis was significantly prolonged when induced after lead nitrate administration. Thus the in vitro inhibition of drug metabolizing activities is accompanied by enhanced sensitivity to the pharmacological action of pentobarbital.

Since sulphydryl groups are involved in the integrity of the mixed function oxidase system, lead could exert a direct action on drug metabolizing enzymes[6]. This possibility has been checked by adding lead nitrate to the incubation mixtures prepared for the assay of demethylating and hydroxylating activities of control rats. The concentrations of lead found in the post-mitochondrial fraction of treated animals were not sufficient to inhibit enzyme activities. Therefore, since lead was inactive in vitro, it can be assumed that binding to functional-SH groups is not responsible for the impairment of drug metabolism in acute lead poisoning.

It appears likely that the decreased capacity of the mixed function oxidase system results from the reduced availability of cytochrome P-450. Lead could exert its action in vivo by decreasing the synthesis and/or increasing the degradation of this hemoprotein.

Indirect evidence for such a mechanism of action was obtained by measuring δ-aminolevulinic acid dehydrase in blood and liver of lead-poisoned rats. The activity was practically absent in the first 2 days after the animals had been treated with lead nitrate (100 μmoles/kg). On the 3rd day the level of δ-aminolevulinic acid dehydrase was only 28% of its normal value and increased to 54% on the 5th day. Since this enzyme activity may be reduced to approximately $^1/_3$ the normal value before heme synthesis is disturbed[10], it can be deduced that inhibition of heme synthesis in lead-poisoned rats occurred during the first 2 to 3 days after lead administration. As a consequence, the biosynthesis of liver hemoproteins should also be inhibited during the same period of time. The half-life of cytochrome P-450 is only 22 h[20]. A prolonged impairment of its synthesis will therefore result in reduced levels unless its degradation was concomitantly lowered.

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The experimental results obtained in this investigation are in good agreement with the above proposed mechanism of action. Conclusive evidence will be provided by further experiments, in which the rate of synthesis of cytochrome P-450 in lead-poisoned rats will be measured directly by incorporation of labelled precursors.

Conclusions. Inhibition of heme synthesis in acute lead poisoning is associated with impairment of the mixed function oxidase system. Since the activity of this enzyme system is an index of hepatic detoxifying capacity for a large number of xenobiotics (drugs, pesticides, food additives, etc.), enhanced sensitivity to such foreign chemicals should be expected to occur in lead-poisoned organisms. Simultaneous exposure to moderately toxic organic compounds, either accidentally or for therapeutic purpose, may result in unpredictable adverse effects.

Zusammenfassung. Eine Verminderung der Konzentration von Cytochrom P-450 sowie der Aktivität von arzneimittelabbauenden Enzymen tritt in der Rattenleber nach Bleivergiftung auf. Intensität und Dauer sind dosisabhängig (10–100 µmol $Pb(NO_3)_2$ pro kg Körpergewicht).

P. SCOPPA, M. ROUMENGOUS and W. PENNING

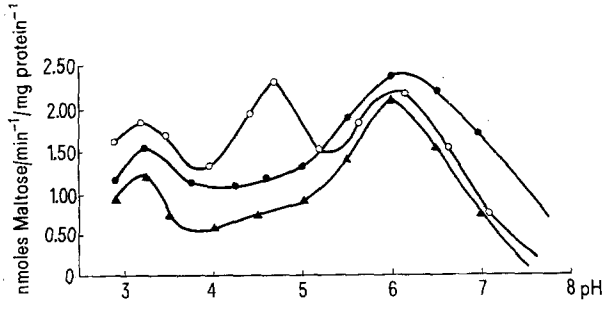
Biology Group, Euratom Joint Research Center, I–21020 Ispra (Italy), 30 January 1973.

α-1,4-Glucosidase Activity in Leucocytes and Lymphocytes of 2 Adult Patients with Glycogen-Storage Disease Type II, (Pompe's Disease)

The glycogenosis independently published by POMPE[1], BISCHOFF[2] and PUTSCHER[3] in 1932, was shown by HERS[4] in 1963 to be due to the absence of α-1,4-glucosidase active at acid pH ('acid maltase') (E.C.3.2.1.20).

In nearly all cases of POMPE's disease cardiac failure leads to death within the first year of life. However, in the last few years, there have been a number of descriptions of patients who reach adolescence or who develop symptoms only at that time[5–18]. Only 4 of these adult patients showed signs of cardiac failure.

In most of the cases, where the α-1,4-glucosidase activity was studied also in leucocytes, no activity at pH4 could be detected in the white blood cells of the babies. But in some of the adult patients with absence of acid maltase in the muscles the α-1,4-glucosidase activity in leucocytes at pH 40 was found to be within the normal range[13, 19].



Variation of α-1,4-glucosidase activity of leucocytes with pH ○—○, Control; ●—●, A.M.; ▲—▲, M.M.

As we have seen in former studies (SEILER and KELLETER[20], unpublished results) that in leucocytes and lympho-

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α-1,4-glucosidase activity (nmoles maltose hydrolyzed/mg protein/min) in leucocytes and lymphocytes of 2 patients with Pompe's disease

	pH 4.0		pH 4.5	
	Leucocytes	Lymphocytes	Leucocytes	Lymphocytes
Controls (n = 20)	2.0 ± 0.7	1.3 ± 0.8	3.0 ± 1.0	1.9 ± 0.8
M.M.	0.6	0.7	0.7	0.7
A.M.	1.1	1.4	1.2	1.4

Leucocytes and lymphocytes were prepared according to[20]. Conditions of assay see[20].