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

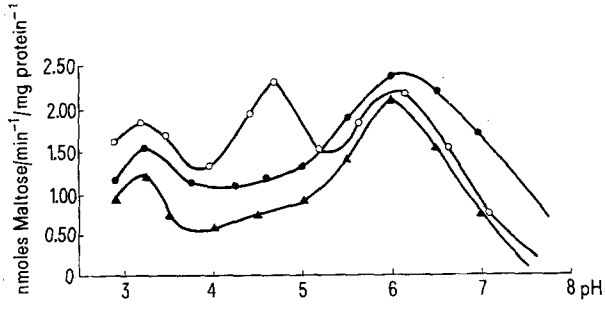
Biology Group, Euratom Joint Research Center, I–21020 Ispra (Italy), 30 January 1973.

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-

[1] J. C. POMPE, Ned. Tijdschr. Geneesk. *76*, 304 (1932).
[2] G. BISCHOFF, Z. Kinderheilk. *52*, 722 (1932).
[3] W. PUTSCHAR, Beitr. path. Anat. *90*, 222 (1932).
[4] H. G. HERS, Biochem. J. *86*, 11 (1963).
[5] V. COURTECUISSE, P. ROYER, R. HABIB, C. MONNIER and J. DEMOS, Archs fr. Pédiat. *22*, 1153 (1965).
[6] H. ZELLWEGER, B. I. BROWN, W. F. MCCORMICK and J. TU, Annls paediat. *205*, 413 (1965).
[7] F. ISCH, J. G. JUIF, R. SACREZ and F. THIEBAUT, Pediatrie *21*, 71 (1966).
[8] H. L. SMITH, L. D. AMICK and J. B. SIDBURY, Am. J. Dis. Child. *111*, 475 (1966).
[9] J. BADOUAL, H. LESTRADET, J. L. VILDE and J. P. PLOUSSARD, Sem. Hôp. Paris (Ann. Pédiat.) *43*, (1427 (1967).
[10] J. SMITH, H. ZELLWEGER and A. K. AFIFI, Neurology, Minneap. *17*, 537 (1967).
[11] H. G. HERS and F. VAN HOOF, in *Carbohydrate Metabolism and its Disorders* (Ed. F. DICKENS; Academic Press, Londen-New York 1968), p. 266.
[12] A. G. ENGEL and A. J. D. DALE, Mayo Clin. Proc. *43*, 233 (1968).
[13] P. HUDGSON, D. GARDNER-MEDWIN, M. WORSFELD, R. J. T. PENNINGTON, J. N. WALTON, Brain *91*, 435 (1968).
[14] K. F. SWAIMAN, W. R. KENNEDY and H. S. SAULS, Archs Neurol. Chicago *18*, 642 (1968).
[15] A. G. ENGEL, in *Muscle Diseases* (Eds. J. N. WALTON, V. CANAL and G. SCARLATO; Excerpta Medica, Amsterdam 1970), p. 236.
[16] N. CANAL, G. SCARLATO, L. FRATTOLA, G. PELLEGRINI and F. CORNELIO, *Actualités de Pathologie Neuro-Musculaire* (Ed. Serratrice; Expansion Scientifique, Paris 1971).
[17] A. G. ENGEL, Brain *93*, 599 (1970).
[18] A. G. ENGEL and M. R. GOMEZ, J. Neurol. Neurosurg. Psychiat. *33*, 801 (1970).
[19] B. ILLINGWORTH BROWN and H. ZELLWEGER, Biochem. J. *101*, 166 (1966).
[20] R. KELLETER and D. SEILER, Clin. chim. Acta *42*, 57 (1972).

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].

cytes the α-1,4-glucosidase activity varies with the pH-value, having maxima at pH 3.2, 4.5 and 6.2, we measured the variations of α-1,4-glucosidase activity with pH in 2 cases of POMPE's disease in adults.

Ensuring the diagnosis of POMPE's disease, we could not find α-1,4-glucosidase activity at pH 4,0 in the muscles obtained from the two male, non-related patients. The clinical features will be published elsewhere.

As can be seen from the Table, we found α-1,4-glucosidase activity at pH 4.0 and 4.5 in both the lymphocytes and leucocytes of the two patients. At these pH-values the α-1,4-glucosidase activity was measured by ILLINGWORTH-BROWN and ZELLWEGER[19] and by HUDGSON et al.[13]. The differences were more pronounced for the leucocytes (mixture of granulocytes and lymphocytes) than for the lymphocytes alone.

Studying the variation of activity with pH we obtained the results shown in the Figure. Only the curves obtained for the leucocytes are presented, but for the lymphocytes we obtained a similar picture. Perhaps the α-1,4-glucosidase activity of the leucocytes and the lymphocytes of the two patients with POMPE's disease at pH 4.0 and 4.5 may be explained as residual activity of the enzymes active at pH 3.2 and 6.2, whereas the enzyme with maximum activity at pH 4.5 seems to be absent or at least diminished in the white blood cells. In the muscles there was no activity at pH 4.0.

The results presented suggest that determination of the variation of α-1,4-glucosidase of leucocytes and lymphocytes with the pH of the assay is a useful tool in the diagnosis of POMPE's disease, especially when a muscle biopsy is rejected.

Zusammenfassung. Die Messung der Aktivität von α-1,4-glucosidase in Lymphozyten und Leukozyten in Abhängigkeit vom pH ergab bei 2 erwachsenen Patienten mit POMPE'scher Erkrankung Aktivitätsmaxima bei pH 3.2 und 6.2. Bei den Kontrollpersonen wurde ein weiteres Maximum bei pH 4,5 gefunden.

D. SEILER[21], R. KELLETER, H. W. KÖLMEL and R. HEENE

Medizinische Universitäts-Poliklinik, Neurologische Universitätsklinik, Hospitalstrasse 3, D-69 Heidelberg (Germany); and Universitäts-Nervenklinik, Marburg (Germany), 23 January 1973.

[21] Acknowledgments. I am indebted to Miss S. BAUER, Miss I. GELDMACHER and Miss R. KIPPHAN for skilful technical assistance.

Analysis of Vasodilatation in the Submaxillary Gland Using Potentiators of Acetylcholine and Kinins

The problem of the resistance of certain parasympathetic nerve effects to inhibition by atropine is unexplained. Perhaps the most classical example is the vasodilatation in the submaxillary gland of the cat and other mammals, which accompanies stimulation of the parasympathetic nerve, the chorda tympani. This has led to views that mechanisms other than cholinergic ones mediate the vasodilatation. Thus, HILTON and LEWIS[1-3] concluded that the enzyme kallikrein, via the release of kinin, is the mediator of this vasodilatation in the salivary and other glands. SCHACHTER et al.[4-6], however, hold the view that kallikrein plays little or no role, and suggest that this parasympathetic effect may be cholinergic despite its resistance to atropine. The possibility that it is mediated by an unknown neurotransmitter is difficult to exclude absolutely; in fact, it has recently been suggested[7] that the parasympathetic atropine-resistant contraction of the urinary bladder is due to the release of a purine nucleotide, possibly adenosine triphosphate.

A classical experiment demonstrating that acetylcholine is a parasympathetic transmitter at least to some cellular elements of the submaxillary gland was done in a simple but dramatic way by BABKIN et al.[8]. They showed that stimulation of the chorda tympani nerve in an eserinized cat produced a fall in the systemic arterial blood pressure after a delay which corresponded to the circulation time. This hypotensive effect was obtained only in the presence of eserine and was prevented by prior injection of atropine.

A group of peptides has recently been synthesized wich potentiates markedly the effects of kinins[9,10]. It now seemed possible to us, therefore, to perform experiments similar to those done by BABKIN et al.[8], but using a kinin potentiator, as well as eserine. Although we found one of these new compounds to be more effective in potentiating the hypotensive effect of bradykinin than eserine was in potentiating that of acetylcholine, we were unable to demonstrate even a trace of bradykinin release during stimulation of the chorda tympani nerve.

Methods. Cats weighing 2.6 to 4.8 kg were anaesthetized with chloralose (60 mg kg^{-1} i.v.). Nerve stimulation, collection of saliva, and blood pressure measurements were carried out as described previously[8]. Drugs were given either close arterially via a lingual artery, or i.v. via a femoral vein. Blood flow was measured via a forced convection flowmeter with a probe in an external jugular vein[11]. Heparin (2 to 10 mg/kg^{-1} i.v.) was given at the beginning of the experiment with further doses as necessary. Bradykinin potentiators were the pentapeptide (PCA-Lys-Trp-Ala-Pro) and the nonapeptide (PCA-Trp-Pro-Arg-Pro-Gin-Ileu-Pro-Pro). The latter was much more effective and was therefore used in 3 of the 4 experiments. The following drugs were used: acetylcholine choloride, atropine sulphate, eserine sulphate and heparin

[1] S. M. HILTON and G. P. LEWIS, J. Physiol. *128*, 235 (1955a).
[2] S. M. HILTON and G. P. LEWIS, J. Physiol. *129*, 253 (1955b).
[3] S. M. HILTON and G. P. LEWIS, J. Physiol. *134*, 471 (1956).
[4] K. D. BHOOLA, J. MORLEY, M. SCHACHTER and L. H. SMAJE, J. Physiol. *179*, 172 (1965).
[5] J. MORLEY, M. SCHACHTER and L. H. SMAJE, J. Physiol. *187*, 595 (1966).
[6] S. BEILENSON, M. SCHACHTER and L. H. SMAJE, J. Physiol. *199*, 303 (1968).
[7] G. BURNSTOCK, B. DUMSDAY and A. SMITHE, Br. J. Pharmac. *44*, 451 (1972).
[8] B. P. BABKIN, A. ALLEY and G. W. STAVRAKY, Trans. R. Soc. Can. *26*, Section V, 89 (1932).
[9] S. H. FERREIRA and M. ROCHA E SILVA, Experientia *21*, 347 (1965).
[10] R. J. FREER and J. M. STEWART, Cienc. Cult. S. Paulo *23*, 539 (1971).
[11] E. KARPINSKI, Ph. D. Thesis, University of Alberta (1971).