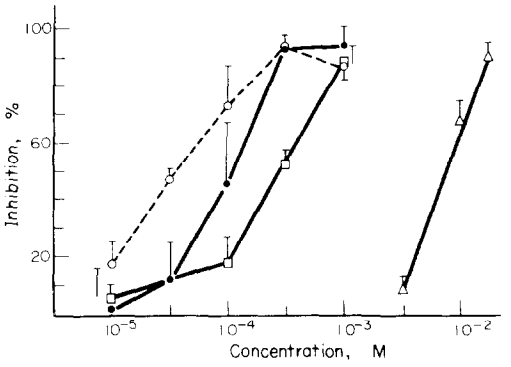

Fig. 2. The inhibitory effects of zinc, lidocaine and complexes between them on rat mast cell histamine release induced by compound 48/80 (0.5 μg/ml). Mean value (± S.D.) from 4–5 experiments are expressed as per cent inhibition. For each experiment 2–3 rats were used. O- -O zinc-lidocaine ionic complex ($ZnCl_4/HLid_2$); ●- -● zinc-lidocaine coordination complex ($ZnLidCl_2$); □- -□ zinc chloride; △ —△ lidocaine hydrochloride.

The potency of the complexes may arise from close binding to the cell membrane; a slow continuous release of zinc ions at this strategic position could favour membrane stability [11, 15]. A possible interaction of compound 48/80 with zinc in the complexes should also be considered. However, earlier experiments with zinc [10] in which inhibition of histamine release was higher when mast cells were preincubated with zinc before compound 48/80 addition than when compound 48/80 was preincubated with zinc before an addition of mast cells rather exclude the direct inactivation of compound 48/80 by zinc. Planned studies of the effect of zinc-lidocaine complexes on antigen-induced histamine release in anaphylaxis may help to clarify this field.

Department of Biogenic Amines WACŁAW KAZIMIERCZAK
Institute of Pharmacology Cracow BLANDYNA ADAMAS
Polish Academy of Sciences CZESŁAW MAŚLIŃSKI
90-136 *Łódź, Narutowicza* 60
Poland

REFERENCES

1. B. Högberg and B. Uvnäs, *Acta physiol. scand.* **48**, 133 (1960).
2. B. Diamant and B. Uvnäs, *Acta physiol. scand.* **53**, 315 (1961).
3. W. Kazimierczak and C. Maśliński, *Agents and Actions* **4**, 1 (1974).
4. W. Kazimierczak, *Acta physiol. pol.* **25**, 609 (1974).
5. S. Kubiak, S. Słomka, W. Kazimierczak and C. Maśliński, *Balneol. Pol.* (in press).
6. J. C. Foreman and J. L. Mongar, *Nature, New Biol.* **103**, 255 (1972).
7. M. Chvapil, *Life Sci.* **13**, 1041 (1973).
8. R. Keller and E. Sorkin, *Experientia* **26**, 30 (1970).
9. H. Fiedler, H. Hahn von Dorsche, P. Fehrman and R. Sulzmann, *Histochemie* **24**, 150 (1970).
10. W. Kazimierczak and C. Maśliński, *Agents and Actions* **4**, 320 (1974).
11. M. Chvapil, J. N. Ryan and C. F. Zukoski, *Proc. Soc. exp. Biol. Med.* **140**, 642 (1972).
12. W. Kazimierczak, M. Peret and C. Maśliński, *Biochem. Pharmac.* **25**, 1747 (1976).
13. A. R. Johnson and N. C. Moran, *Proc. Soc. exp. Biol. Med.* **123**, 886 (1966).
14. C. Code, F. F. C. McIntire and D. Glick, *Meth. Biochem. Analysis* **3**, 49 (1959).
15. A. Albert, *Selective Toxicity*, p. 338. Methuen, London (1968).

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Glycolytic metabolites and adenosine triphosphate in skeletal and cardiac muscle of rats after clofibrate feeding

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An acute muscular syndrome was reported in patients treated with ethylchlorophenoxyisobutyrate [1], including myalgia, cramps, weakness and stiffness. This seems to be an uncommon side effect of clofibrate therapy [2], possibly correlated to higher doses [1] and free serum levels of the drug [3]. Even mild muscular effort may accentuate these side effects [4]. Similar symptoms were seen in patients suffering from a heriditary insufficiency of muscle phosphorylase, which causes an inadequate glucose supply from glycogen during muscle contraction [5]. Clofibrate reduces liver glycogen levels in animals [6, 7] and modifies the content of glycolytic metabolites in rat liver [8, 9]. In addition myotonia was induced in rats by clofibrate [10]. This could suggest an interference of clofibrate with glucose metabolism of muscle.

Female rats, Wistar strain, were used throughout. They were kept on a diet with 0.25% (w/w) clofibrate. (The diet was a gift of the ICI Industrial Company, Macclesfield, England). The mean weight of the animals was 210 ± 15 g and 222 ± 25 g (±S.E.) after 3 or 6 weeks of clofibrate feeding. The values of the control groups were 207 ± 15 g and 238 ± 25 g respectively.

Muscle of the hind leg and the whole cardiac muscle were freeze-clamped. The detailed analytical procedure is described elsewhere [9]. The U-test of Wilcoxon, Man and Whitney was used for statistical analysis.

A decreased glycogen content of rat skeletal muscle was reported also by Miyazawa *et al.* [8]. Lower glycogen values are also found in the cardiac muscle after clofibrate exposure [17.7 ± 1.3 μmoles glycosyl-units g wet wt^{-1} (mean ± S.E.M.), $n = 10$ in the experimental group, 25.1 ± 1.9 μmoles, $n = 9$ in the control group). The decrease of 29.5 per cent was significant ($P < 0.05$). The weight of the hearts did not differ in control (0.654 ± 0.14 g, mean ± S.E.) and the clofibrate group (0.645 ± 0.07 g). The mechanism of glycogen decrease during clofibrate treatment in rats is not yet completely understood. Some data are available to support a reduced glycogen synthesis [11]. Incorporation of ^{14}C-labelled glucose into glycogen is reduced in liver slices of clofibrate treated rats [11] and dogs [7]. The enzyme glycogen synthetase (Glycogen-UDP glycosyl-transferase EC 2.4.1.11] is activated by insulin via the modification of the protein kinase [12]. Immunoreactive insulin was reported to be reduced

Table 1. Glycolytic metabolites in skeletal muscle (μmoles g wet wt^{-1}) after 3 weeks of feeding clofibrate (0.25% w/w). Concentrations represent mean values + S.E.M. Glycogen in glycosyl-units

	Glycogen	G-6P	F-6-P	$F_{1-6}P$	PEP	Pyr	Lac
Control ($n = 10$)	30.72 ± 2.22	0.545 ± 0.05	0.105 ± 0.011	0.201 ± 0.018	0.023 ± 0.005	0.064 ± 0.008	1.42 ± 0.13
Clofibrate ($n = 11$)	22.01 ± 1.85	0.421 ± 0.04	0.077 ± 0.005	0.129 ± 0.012	0.015 ± 0.002	0.062 ± 0.009	1.07 ± 0.09
Change in per cent of control	−28.4	−21.8	−26.7	−35.8	−34.8	−3.1	−24.6
P-value	<0.01	<0.025	<0.05	<0.005	n.s.	n.s.	<0.05

during clofibrate treatment in rats [13]. On the other hand the reduced level of glucose 6-phosphate (Table 1) could induce lower glycogen level in skeletal muscle via deinhibition of phosphorylase *b* (α-Glucan phosphorylase EC 2.4.11) [14]. In addition to glycogen many of the glycolytic metabolites in rat skeletal muscle are reduced by clofibrate (Table 1). Similar alterations were described in rat liver [9, 10]. The lactate:pyruvate ratio of 17.3 in the clofibrate group (control group 22.0) indicates a lower NADH:NAD quotient as found in rat liver [15]. The lower substrate levels may indicate an enhanced flux of substrates through the glycolytic pathway [9]. Clofibrate induced a higher rate of [1-^{14}C]glucose oxidation to CO_2 [7], but not from [6-^{14}C]glucose in liver slices [7, 11]. Data about the rate of glycolysis in skeletal muscle during clofibrate exposure are not available so far. However, the reduced levels of glucose metabolites in muscle may possibly refer to insufficient substrate supply for anaerobic ATP regeneration during muscle contraction. To study this assumption the ATP-level in muscle during clofibrate treatment was measured.

No significant changes were found in both tissues after 3 weeks of clofibrate exposure. However, a reduction of ATP from 5.94 ± 0.13 μmoles g wet wt^{-1} (mean ± S.E.M., $n = 9$) to 4.84 ± 0.14 was measured in skeletal muscle after 6 weeks. The decrease of 18.5 per cent was significant ($P = <0.001$). Similar, but insignificant change of ATP was seen in cardiac muscle. The suggestion made above that an insufficient substrate supply may induce a lower ATP-level seems to be inconclusive, since the decrease of glycogen and other glycolytic metabolites precedes the decrease of ATP of weeks.

Clofibrate inhibits state 3 phosphorylation in isolated rat liver mitochondria [16, 17] and palmitate utilisation in isolated heart mitochondria *in vitro* [18]. An altered energy production of liver or muscle mitochondria isolated from clofibrate treated rats could not be demonstrated [19] and clofibrate in a concentration of 0.25% in the diet do not penetrate into liver cells [6]. More data are requested about the influence of clofibrate on glucose metabolism and oxidative phosphorylation of muscle *in vivo*.

SUMMARY

The influence of chronic clofibrate feeding on glucose metabolites and ATP was investigated in skeletal and cardiac muscle of rats. After 3 weeks of clofibrate exposure glycogen and all hexose-phosphate intermediates were found to be decreased in skeletal muscle. Cardiac glycogen content was reduced after clofibrate feeding too. In contrast, after 6 weeks only lower ATP-levels were induced by clofibrate feeding. Similar but insignificant data were found in cardiac muscle.

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Medizinisch Klinik II
Klinikum Grosshadern
Universität of München
Marchioninistrasse 15
8000 *München* 70, *Germany*

J. WILKENING
F. WOLPERT
P. SCHWANDT

REFERENCES

1. T. Langer and R. Levy, *New Engl. J. Med.* **279**, 856 (1968).
2. A. F. Smith, W. G. Macfie and M. F. Oliver, *Br. Med. J.* **2**, 86 (1970).
3. J. F. Bridgman, S. M. Rosen and J. M. Thorp, *Lancet* **2**, 506 (1972).
4. D. Geltner, M. Chaco and M. Shapiro, *Postgrad. Med. J.* **51**, 184 (1975).
5. B. McArdle, *Clin. Sci.* **10**, 13 (1951).
6. D. S. Platt and J. M. Thorp, *Biochem. Pharmac.* **15**, 915 (1966).
7. J. H. Gans and M. R. Cater, *Biochem. Pharmac.* **20**, 3321 (1971).
8. S. Miyazawa, T. Sakurai, M. Imura and T. Hashimoto, *J. Biochem.* **78**, 1171 (1975).
9. J. Wilkening and P. Schwandt, *Horm. Metab. Res.* **9**, 132 (1977).
10. S. H. Dromgoole, D. S. Campion and J. B. Peter, *Biochem. Med.* **14**, 238 (1975).
11. D. Zakim, R. S. Paradini and R. H. Herman, *Biochem. Pharmac.* **19**, 305 (1970).
12. C. Villar-palasi and J. I. Wenger, *Fedn Proc.* **26**, 563 (1967).
13. A. S. Weis, H. M. Tepperman and J. Tepperman, *Endocrinology* **93**, 504 (1973).
14. H. E. Morgan and A. Sarmeggiani, *J. biol. Chem.* **239**, 2440 (1964).
15. D. S. Platt, B. L. Cockrill, *Biochem. Pharmac.* **15**, 927 (1966).
16. C. R. Mackerer and J. R. Haettinger, *Biochem. Pharmac.* **23**, 3331 (1974).
17. A. I. Cederbaum and E. Rubin, *Biochem. Pharmac.* **23**, 1985 (1974).
18. H. H. Stein and J. Cohen, *Fedn Proc.* **35**, 746 (1976).
19. A. I. Cederbaum, T. V. Madhavan and E. Rubin, *Biochem. Pharmac.* **25**, 1285 (1976).