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# BLOOD-GLUCOSE-LOWERING ACTIVITY OF 2-(3-PHENYLPROPOXYIMIDO)-BUTYRATE (BM 13.677)

H. F. KÜHNLE, H. P. WOLFF, F. H. SCHMIDT and R. REITER*
Boehringer Mannheim GmbH, Dept. Medical Research, D-6800 Mannheim 31, Federal Republic of Germany

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**Abstract**—A single oral or intraperitoneal application of 2-(3-phenylpropoxyimido)-butyrate (BM 13.677) resulted in a dose-dependent blood-glucose-lowering effect in fasted guinea-pigs. The threshold dose and the $EC_{50}$ were estimated as 25 mg/kg and 63 mg/kg, respectively, which is between that of the biguanides phenformin and metformin. A rise in blood lactate concentrations was observed only at high doses of BM 13.677, but was not related to an irreversible metabolic inhibition. Among several rodent species studied the potency of the drug decreased in the order guinea-pig ≫ mouse > rat = rabbit. Inhibition of hepatic gluconeogenesis by the drug was demonstrated in the perfused liver or hepatocytes of guinea-pigs. Inhibition of glucose production by the perfused liver in the presence of 0.1 mM BM 13.677 was dependent on the substrate and decreased in the order: lactate > pyruvate > alanine ≫ propionate > glycerol = fructose. This suggests a specific interaction of the drug with a mitochondrial key reaction of gluconeogenesis. Stimulation of glucose oxidation in rat diaphragm by the compound ($EC_{50}$ = 0.85 mM) suggests that besides inhibition of gluconeogenesis also extrahepatic effects contribute to the blood-glucose-lowering effects of the drug.

Diabetes mellitus is characterized by a deranged glucose metabolism. In type-I diabetes [1] this is the result of a complete lack of pancreatic insulin production, while an impaired sensitivity of tissues towards insulin is one characteristic of type-II diabetes [2]. Rationales in treatment of the latter include a restoration of the sensitivity of peripheral tissues towards insulin, an inhibition of hepatic gluconeogenesis and/or enteral glucose resorption.

The biguanides [3, 4] are a class of compounds that exert a number of these activities. Suppression of hepatic gluconeogenesis [5, 6], inhibition of enteral glucose resorption [7, 8] and stimulation of peripheral glucose uptake [9, 10] have been reported. Biguanides therapy by itself or in combination with sulfonylureas formed a powerful approach in the treatment of type-II diabetes [11]. However, an increased incidence of non-responsive lactaemia [12, 13] during biguanide treatment suggested, that under certain conditions, the drug caused an inhibition of oxidative metabolism [14, 15]. This severely compromised their therapeutical usefulness and led to a banning of the most potent biguanides like phenformin, as well as restrictions in the use of others [16].

Nevertheless, the interesting therapeutical principle represented by the biguanides has fueled the development of new antihyperglycaemic drugs that share therapeutical benefits, but lack the side effects of the biguanides.

2-(3-Phenylpropoxyimido)-butyrate (BM 13.677) is a representative of a new class of hypoglycaemic compounds with oxime structure, which have been synthesized in our laboratories. In the present study, we characterize the blood-glucose-lowering effect of the compound with phenformin and metformin serving as references.

* Address for correspondence: Boehringer Mannheim GmbH, Diabetes Research (FM-1 D), Sandhofer Strasse 116, D-6800 Mannheim 31, F.R.G.

## MATERIALS AND METHODS

*Animals*. Male guinea-pigs (300–400 g) and Chinchilla rabbits (2.2–2.4 kg) were purchased from Meckel (Laubach, F.R.G.). Lewis rats, weighing 200–300 g and mice (strain: NMRI/Han Crl BR) weighing 20–28 g were from Savo-Invanovas (Kisslegg, F.R.G.). and Charles River WIGA (Sulzfeld, F.R.G.), respectively. The animals were housed in temperature- and humidity-controlled quarters with artificial daylight from 6:00 a.m. to 6:00 p.m. They were fed pellet diets, supplemented with the appropriate vitamin premix, and water *ad lib*.

*Chemicals*. Sodium 2-(3-phenylpropoxyimido)-butyrate (BM 13.677) and metformin hydrochloride were synthesized in the Chemistry Department of Boehringer Mannheim GmbH. Phenformin hydrochloride was purchased from Hoechst AG (Frankfurt, F.R.G.). [U-$^{14}$C]Glucose (sp. radioact. 37 MBq/mmol) was obtained from NEN (Dreieich, F.R.G.). All other chemicals and biochemicals used, including enzymes and coenzymes were of the highest purity commercially available; most were supplied by Boehringer Mannheim GmbH.

In vivo *experiments*. Rats, mice and rabbits were fasted 24 hr, and guinea-pigs were fasted 48 hr prior to the experiments; water was *ad lib*. When the blood-glucose-lowering activities were studied, the drugs were given either intraperitoneally, intravenously or orally in the doses indicated. A 0.5% tylose suspension or saline served as controls. At the intervals indicated, blood samples were collected from the ear vein (10–20 μL) and assayed for glucose

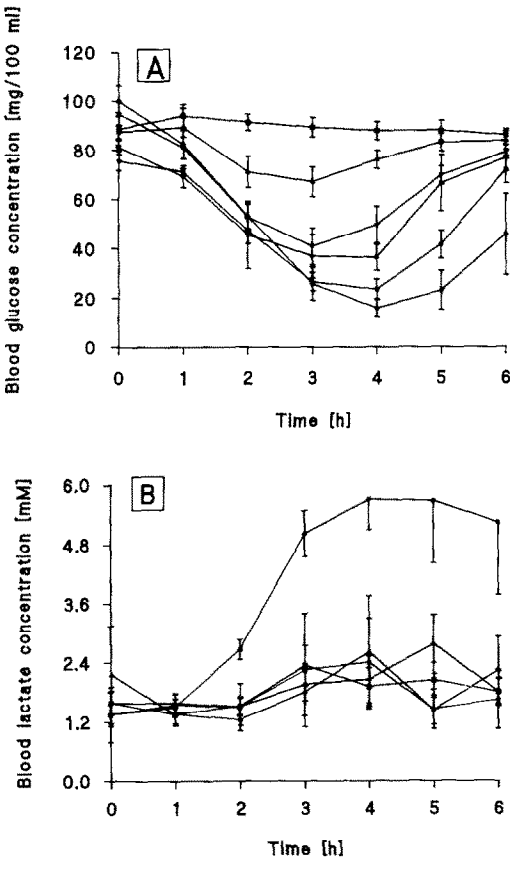


Fig. 1. Effects of a single oral application of BM 13.677 on blood glucose (panel A) and lactate (panel B) concentrations in fasted guinea-pigs: (●) control, (■) 25 mg/kg, (▲) 50 mg/kg, (▼) 75 mg/kg, (★) 100 mg/kg, (◆) 150 mg/kg (N = 6).

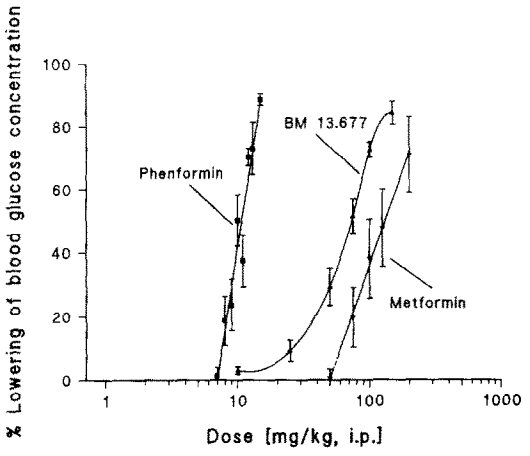


Fig. 2. Dose dependence of maximal blood-glucose-lowering effect of a single i.p. dose of BM 13.677, phenformin or metformin in fasted guinea-pigs (N = 6). Fasting blood glucose concentrations were 91.7 ± 3.1 mg/100 mL.

[17]. For the determination of blood lactate [18] concentrations 50–100 μL of capillary blood were obtained and immediately assayed. In some experiments, severe hypoglycaemia was provoked by intraperitoneal injection of 300 mg/kg BM 13.677. One group (N = 6) received only the drug; a second group received in addition to the drug also 0.5 g/kg glucose after 0.25, 3, and 5 hr, while a third group served as control. Mortality was monitored over a 24 hr observation period. In the dose–effect curves, maximal lowering of blood glucose concentrations as compared to the control group was calculated as a function of the administered dose.

In vitro *experiments*. Glucose production was studied in the non-recirculating, hemoglobin-free perfusion of the liver of guinea-pigs, which had been fasted for 48 hr [19]. Lactate (5 mM), pyruvate (2 mM), alanine (3.5 mM), fructose (5 mM), propionate (10 mM) or glycerol (5 mM) were added as substrates to the serum albumin-free perfusate. The effluent was fractioned in 5 min intervals and assayed for glucose. Hepatocytes were isolated by collagenase treatment according to Krebs *et al.* [20] from the livers of guinea-pigs, which had been fasted for 48 hr. Hepatocytes (10 mg/mL) were suspended in Erlenmeyer flasks at a final volume of 1 mL Krebs–Ringer bicarbonate buffer, pH 7.4, supplemented with 4% essentially fatty acid-free bovine serum albumin. The flasks were flushed with carbogen and incubated at 37° in a shaking water bath (80 strokes/min). The reaction was terminated by sedimentation of the cells, and glucose was determined in the supernatant.

Hemidiaphragms were prepared from rats (200–250 g), which had been fasted for 24 hr. Following a 60 min preincubation in Krebs–Ringer bicarbonate buffer supplemented with 4% bovine serum albumin, [U-$^{14}$C]-labelled glucose was added and incubated at 37° in a shaking water bath (80 strokes/min) over 60 min in a sealed flask. Carbon dioxide released from glucose was trapped in a hyamine containing center well following addition of 200 μL 1 M sulfuric acid and was quantitated by liquid scintillation counting.

*Statistics*. All data are given as mean ± SE; significance was calculated according to Wilcoxson.

## RESULTS

Oral application of a single dose of BM 13.677 to fasted guinea-pigs resulted in a dose dependent fall in blood glucose concentrations (Fig. 1A). Maximal effects were reached within 3–4 hr after drug administration. The threshold dose and the $EC_{50}$ were estimated as 25 and 63 mg/kg, respectively. As can be seen in Fig. 1B, a roughly four-fold rise in blood lactate concentrations was only detectable following application of 150 mg/kg BM 13.677, e.g. at doses causing severe hypoglycaemia. When increasing the dose to 300 mg/kg BM 13.677, five of a total of six animals died within 6 hr after drug administration. Intraperitoneal injection of 0.5 g/kg glucose 0.25, 3 and 5 hr after drug administration completely protected the guinea-pigs, suggesting that the hypoglycaemic effect of the compound was not

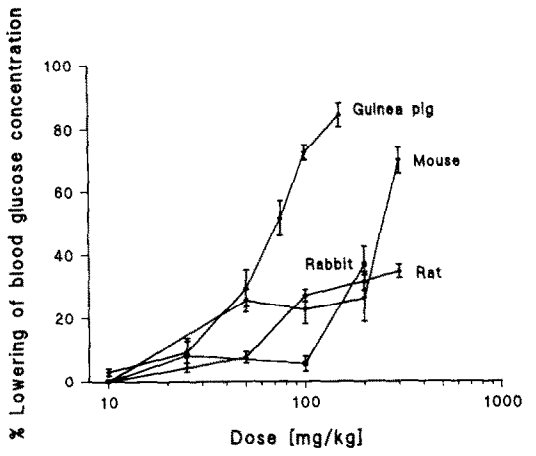


Fig. 3. Maximal lowering of blood-glucose concentrations in various fasted rodents following a single i.p. injection of BM 13.677 (N = 6). The starting blood glucose concentrations were 75.2 ± 2.4 (rabbit), 78.0 ± 1.2 (rat), 71.5 ± 3.1 (mouse) and 87.5 ± 2.8 mg/100 mL (guinea-pig).

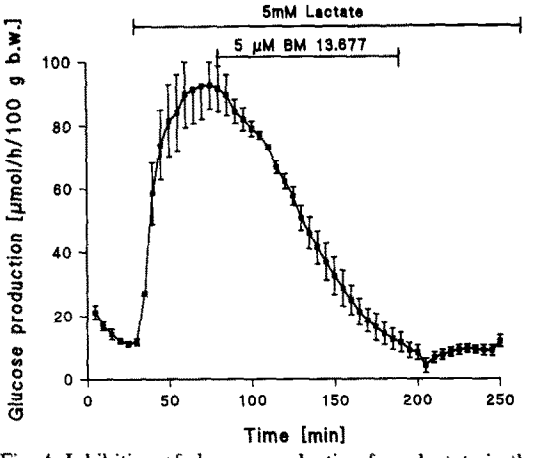


Fig. 4. Inhibition of glucose production from lactate in the perfused guinea-pig liver by 5 µM BM 13.677 (N = 3).

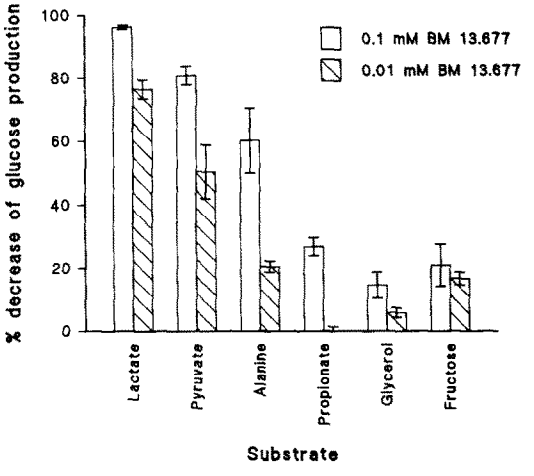


Fig. 5. Dependence of the inhibitory potency of BM 13.677 on glucose production in perfused guinea-pig liver from the glucogenic substrate. All substrates were added to perfusate at 5 mM concentration, except fructose, which was 10 mM (N = 3). The glucose production rates in control incubations were 147.7 ± 4.0 (lactate), 72.5 ± 5.0 (glycerol), 86.4 ± 6.5 (alanine), 234.9 ± 7.5 (fructose), 164.3 ± 15.2 (propionate) and 104.2 ± 4.8 µmol/hr/100 g body weight (pyruvate).

accompanied by an irreversible inhibition of oxidative metabolism at high doses.

Oral or intraperitoneal application of the compound yielded virtually the same blood glucose lowering effects. The dose–effect curve of BM 13.677 in fasted guinea-pigs as compared to phenformin and metformin is shown in Fig. 2. The biguanides are characterized by steeper slopes of their dose–effect curves than the oxime. Compared on an equipotency basis the $EC_{50}$ of BM 13.677 was five to six times higher than that of phenformin ($EC_{50}$ = 11 mg/kg), but two times less than that of metformin ($EC_{50}$ = 125 mg/kg) when the drugs were given intraperitoneally.

The species-dependence of the blood-glucose-lowering effect of BM 13.677 among several rodent species is shown in Fig. 3. The fasted guinea-pig was by far the most sensitive rodent studied but with increasing doses a blood-glucose-lowering effect was observed also in rats, mice or rabbits. Intravenous injection of 200 mg/kg BM 13.677 in rabbits resulted in a 38% fall in blood glucose concentrations over control. Doses of 300 mg/kg i.p. lowered blood-glucose concentrations by 70% and 35% in mice and rats, respectively. The above described effect of BM 13.677 on glucose metabolism is similar to that of the biguanides and, hence, a similar mechanism of action might be expected. Inhibition of hepatic gluconeogenesis and stimulation of glucose oxidation in muscle tissue by biguanides are well known. Therefore, we studied the effect of BM 13.677 on these processes.

Concentrations as low as 5 µM BM 13.677 caused a significant 65% inhibition in glucose production of the perfused guinea-pig liver over control when lactate served as a glucogenic precursor (Fig. 5). When studying hepatic gluconeogenesis from various substrates (Fig. 6) a dependence of the inhibitory effect of BM 13.677 on the nature of the substrate was found. At 10 µM BM 13.677 glucose formation from lactate or pyruvate was reduced by 76% or 50% over control, respectively, while gluconeogenesis from other substrates was only marginally affected. Inhibition by 0.1 mM BM 13.677 as compared to control was most pronounced in the presence of lactate (97%), followed by pyruvate (80%) and alanine (60%). In contrast, glucose formation from fructose, glycerol, or propionate was suppressed to a lesser extent (15–30%) under these conditions.

In hepatocytes of guinea-pigs approximately 10–15-fold higher concentrations as compared to the perfused liver were necessary in the presence of 4 mg/mL serum albumin, in order to achieve similar inhibitory effects on gluconeogenesis. This apparent

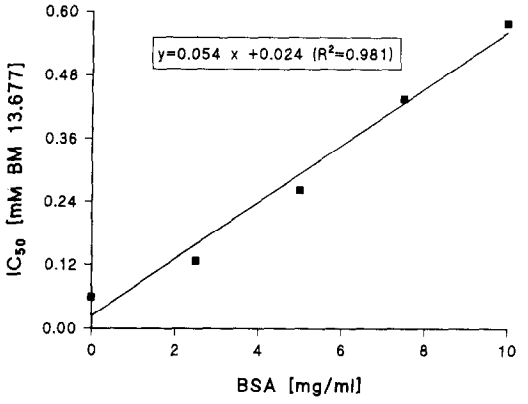


Fig. 6. Dependence of the inhibitory activity of BM 13.677 on gluconeogenesis from addition of bovine serum albumin (BSA) to suspension of guinea-pig hepatocytes using 5 mM lactate as substrate.

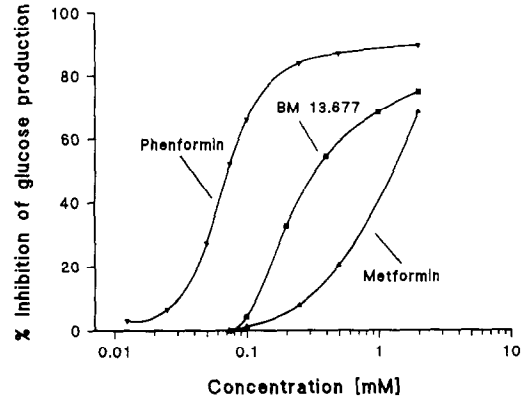


Fig. 7. Concentration dependence of the inhibitory effect of BM 13.677, phenformin or metformin on gluconeogenesis in guinea-pig hepatocytes using 5 mM lactate as substrate (N = 6). The glucose production rate in control incubations was 27.6 ± 0.3 μg/hr/mg protein.

discrepancy was partially due to the binding of the compound to serum albumin as suggested by an increase in inhibitory activity of the compound with decreasing serum albumin concentrations (Fig. 6). A 50% inhibition of glucose production from lactate was found in the presence of 0.45 mM BM 13.677. Compared to phenformin ($IC_{50}$ = 0.075 mM) and metformin ($IC_{50}$ = 1.25 mM) the potency of BM 13.677 in this model was also between that of the two biguanides (Fig. 7). The contribution of a stimulation of peripheral glucose metabolism besides the inhibition of hepatic gluconeogenesis to the blood-glucose-lowering activity of BM 13.677 was studied subsequently using isolated hemidiaphragms of rat as a model muscle tissue. As shown in Fig. 8, basal glucose oxidation rates of 7 nmol/g tissue/hr were stimulated by millimolar concentrations of BM 13.677 up to 34 nmol/g tissue/hr with half-maximal effects being reached in the presence of 0.85 mM BM 13.677.

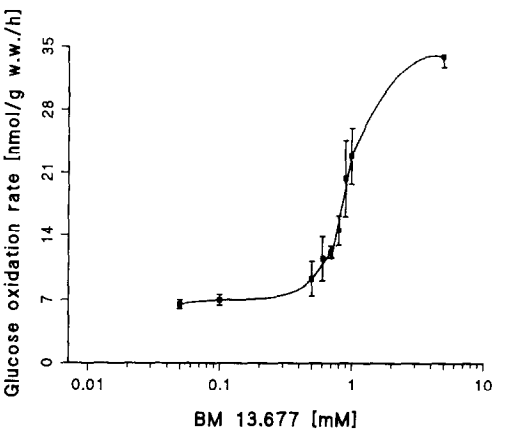


Fig. 8. Concentration dependence of stimulation of glucose oxidation in rat hemidiaphragms by BM 13.677 (N = 4).

## DISCUSSION

The guinea-pig was chosen as an animal model in the study of the blood-glucose-lowering activity of BM 13.677 based on the intrahepatic distribution of key enzymes of gluconeogenesis similar to man. With respect to their pharmacological effects, the oxime and the biguanides show a remarkable resemblance. Firstly, the hypoglycaemic potency of BM 13.677 is between that of phenformin and metformin. Secondly, the oxime shares the pronounced species-dependence of its blood-glucose-lowering effect with the biguanides. As with phenformin, BM 13.677 is a potent blood-glucose-lowering agent in guinea-pigs, and to a lesser extent also in mice, while only weak hypoglycaemic effects are detectable in rats or rabbits. Finally, both compound classes exert their blood-glucose-lowering effect by several mechanisms such as suppression of hepatic gluconeogenesis and stimulation of glucose oxidation in muscle tissue.

However, although the two compound classes interact with the same metabolic pathways, they differ considerably in their mechanism of action. Lactic acidosis caused by an inhibition of oxidative metabolism is a well-known side effect of high doses of biguanides. Although at high doses of BM 13.677 severe hypoglycaemia in guinea-pigs was associated with a rise in blood lactate concentrations the complete protection by repeated doses of glucose against a lethal dose of BM 13.677 strongly suggests that oxidative metabolism remains unaffected under these conditions. Therefore, the increase in blood lactate concentration produced by high doses of BM 13.677 seems to be the consequence of an inhibition of gluconeogenesis rather than an impairment of oxidative phosphorylation.

The dependence of the inhibitory potency of BM 13.677 on the precursors of gluconeogenesis in perfused guinea-pig liver corroborates this view. Fructose or glycerol enter the pathway of gluconeogenesis through cytosolic intermediates and depend on NADH and/or ATP for glucose formation. Therefore, an inhibition of energy metabolism by BM 13.677 must be regarded as unlikely since glucose production from these substrates was only marginally

affected. The pronounced inhibition of hepatic gluconeogenesis by BM 13.677 from lactate, pyruvate, or alanine under these conditions suggests a specific interaction with the mitochondrial key reactions of gluconeogenesis. Since glucose formation from propionate, which is metabolized via succinylCoA to oxaloacetate and thereby circumvents the pyruvate carboxylase reaction, is affected to a much lesser extent, inhibition of the latter enzyme reaction seems the most likely interaction site. A direct inhibition of the enzyme or an effect on cosubstrate availability is conceivable.

Besides inhibition of hepatic gluconeogenesis probably also stimulation of glucose oxidation in muscle tissue contributes to the blood-glucose-lowering activity of the compound. The effects on glucose metabolism in diaphragms or hepatocytes are characterized by a similar concentration-dependence and, hence, may occur in the same dose range. The mechanism by which BM 13.677 stimulates glucose oxidation in diaphragms remains a matter of conjecture. Either an insulin-like, an insulin sensitizing effect, or an interference with the Randle cycle [21], e.g. the mutual regulation of glucose and fatty acid oxidation in muscle or adipose tissue in order to control blood-glucose and fatty-acid concentrations in blood, seem conceivable.

## REFERENCES


1. Eisenbarth GS, Type I Diabetes mellitus. *N Engl J Med* **314**: 1360–1368, 1986.
2. Grill V and Efendic S (Eds.), *Pathogenesis of Non-insulin Dependent Diabetes Mellitus*. Raven Press, New York, 1989.
3. Soeling HD and Ditschuneit H, Die Biguanide. In: *Handbuch des Diabetes Mellitus* (Ed. Pfeiffer EF), Vol. 1, pp. 685–703. Lehmann Verlag, Muenchen, 1969.
4. Beckmann R, Biguanides. In: *Handbook of Experimental Pharmacology* (Ed. Maske H), Vol. 29, pp. 439–596. Springer, Heidelberg, 1971.
5. Meyer F, Ipaktchi M and Clauser H, Specific inhibition of gluconeogenesis by biguanides. *Nature* **213**: 203–204, 1967.
6. Wollen N and Bailey CJ, Inhibition of hepatic gluconeogenesis by metformin. *Biochem Pharmacol* **37**: 4353–4358, 1988.
7. Kruger FA, Altschuld RA, Hollobough SL and Jewett B, Studies on the site and mechanism of action of phenformin. *Diabetes* **19**: 50–52, 1970.
8. Lorch E, Inhibition of intestinal absorption and improvement of oral glucose tolerance by biguanides in the normal and in the streptozotocin-diabetic rat. *Diabetologia* **7**: 195–203, 1971.
9. Pereira JN, Jangaard NO and Pinson ER, Some metabolic effects of phenformin in rat adipose tissue. *Diabetes* **16**: 869–872, 1967.
10. Frayn KN, Adnitt PI, Effects of metformin on glucose uptake by isolated diaphragm from normal and diabetic rat. *Biochem Pharmacol* **21**: 3153–3162, 1972.
11. Gerich JE, Drug therapy-oral hypoglycaemic agents. *N Engl J Med* **321**: 1231–1245, 1989.
12. Alberti KGMM and Nattrass M, Lactic acidosis. *Lancet* **2**: 25–29, 1977.
13. Phillips PJ, Scicchitano R, Clarkson AR and Gilmore HR, Metformin associated lactic acidosis. *Aust NZ J Med* **8**: 281–284, 1978.
14. Caspary WF and Creutzfeldt W, Inhibition of intestinal amino acid transport by blood sugar lowering biguanides. *Diabetologia* **9**: 6–12, 1973.
15. Schaefer G, On the mechanism of action of hypoglycaemia-producing biguanides. A reevaluation and a molecular theory. *Biochem Pharmacol* **25**: 2005–2014, 1976.
16. Kolata GB, The Phenformin ban: is the drug an imminent hazard? *Science* **203**: 1094–1096, 1979.
17. Schmidt FH, Die Bestimmung von Glukose und Fruktose nebeneinander. *Klin Wschr* **39**: 1244–1247, 1961.
18. Gutman I and Wahlefeld AW, L-Lactat. In: *Methoden der Enzymatischen Analyse* (Ed. Bergmeyer HK), p. 1510. Verlag Chemie, Weinheim/Bergstrasse, 1974.
19. Kuehnle HF, Schmidt FH and Deaciuc IV, *In vivo* and *in vitro* effects of a new hypoglycaemic agent, 2-(3-Methylcinnamylhydrazono)-propionate (BM 42.304) on glucose metabolism in guinea pig. *Biochem Pharmacol* **33**: 1437–1444, 1984.
20. Krebs HA, Cornel NV, Lund P and Hems R, In: *Regulation of Hepatic Metabolism* (Eds. Lundquist F and Tygstrup N), p. 126. Munksgaard, Copenhagen, 1975.
21. Randle PJ, Garland PB, Hales CN and Newsholme EA, The glucose fatty-acid cycle. *Lancet* **1**: 785–789, 1963.