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An Experimental Model of Phenformin-Induced Lactic Acidosis in Rats

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Summary. An experimental model of phenformin-induced lactic acidosis was established in rats. Following a subtotal nephrectomy, renal failure developed (serum creatinine 4.5 ± 0.1 mg/100 ml and 2.8 ± 0.1 mg/100 ml on the 1st and 8th post-operative days respectively). Immediately after nephrectomy intra-peritoneal phenformin treatment, 16 mg/day, was commenced. Lactic acidosis developed progressively within 8 days, or earlier in the rats with the most severe renal insufficiency. The metabolic pattern was very similar to that observed in diabetic patients with a biguanide-induced lactic acidosis: on the 8th day, 2 h after the last phenformin injection, blood lactate was 10.8 ± 1.0 mmol/l (controls: 1.50 ± 0.03); pyruvate was 0.56 ± 0.06 mmol/l (controls: 0.10 ± 0.01) and blood pH: 7.00 ± 0.02 (vs 7.34 ± 0.02); 3-hydroxybutyrate was 1.41 ± 0.37 mmol/l (vs 0.32 ± 0.03); acetoacetate: 0.51 ± 0.15 mmol/l (vs 0.17 ± 0.01), and free glycerol: 0.63 ± 0.07 mmol/l (vs 0.14 ± 0.02). Increased concentrations of alanine (1.66 ± 0.26 mmol/l, vs 0.48 ± 0.04 in controls) and low blood glucose levels (23 ± 8 mg/100 ml vs 70 ± 2, after a 12 hours fast) accompanied the lactic acidosis in spite of high glucagon levels (2030 ± 170 pg/ml vs 108 ± 10 in controls) and low insulin/glucagon molar ratio (0.19 vs 6.9 in controls). Normal rats, treated with phenformin at same doses, and nephrectomized rats injected with saline served as controls and remained free of lactic acidosis. Hydroxyphenformin (16 mg/day) injected in nephrectomized rats, was biologically inactive.

Glucose production from ^{14}C-lactate was 425 ± 85 μmol/100 g body wt/h, vs 1050 ± 90 in control animals. Blood lactate specific activity declined more slowly in the lactic acidotic rats than in controls, suggesting that a decrease in lactate utilization contributed to hyperlactataemia more than an increased lactate production.

Key words: Lactic acidosis, phenformin, biguanides.

The occurrence of lactic acidosis in diabetic patients is often associated with antecedent phenformin treatment. Renal failure is often superimposed [1–3] and pharmacological studies have shown that phenformin is to some extent detoxified in the liver and excreted mainly in the urine [4–7]. However, the role of phenformin in lactic acidosis and the eventual intervention of renal failure in accumulation of the drug have been so far suspected rather than proved. The aim of the present work was to describe an animal model for the study of biguanide-induced lactic acidosis.

Methods

1. Experimental Procedures

Adult male Wistar rats (250–300 g) were anaesthetized using thiopental (50 mg/kg IP). Bipolar nephrectomy of the right kidney, removing two-thirds of renal tissue, was performed. A tie placed around the left renal vessels was exteriorized at the lumbar surface, then tightly ligated 3 days later under light ether anaesthesia [8]. Serum creatinine concentration was measured from the 1st to the 8th day after ligation, by a routine method. Phenformin treatment was started in the hours following ligation of the left renal vessels. Doses of phenformin 4 to 40 mg/rat/day were investigated. A single injection of 40 mg/rat was immediately followed by convulsions and death without any significant change of blood lactate and glucose. Daily injection of 4 mg and 8 mg/rat induced a definite rise of blood lactate after two

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weeks of treatment in nephrectomized rats. A dose of 16 mg/rat/day (in 2 ml physiologic saline solution) was finally chosen because of its clear-cut metabolic effects within one week of treatment. Other nephrectomized rats were injected with 2 ml saline, or with 4-hydroxy-phenformin 16 mg/rat/day (kindly supplied by Dr. Beckmann, Chemie Grünenthal GmbH, Stolberg, West Germany). Control rats were sham-operated and similarly injected. Every second day, 2 hours after injections, blood samples (2 ml) were obtained by careful cardiac puncture, under light thiopental anaesthesia (20 mg/kg IP) in an attempt to minimize stress-induced hyperlactataemia. These samples were analyzed for substrates or hormone and pH determinations.

2. Blood Pressure and Temperature Measurements

Arterial blood pressure was measured every 15 min for 3 h after phenformin injection, on the 5th day of treatment. The carotid artery was cannulated under thiopental anaesthesia (50 mg/kg IP) and connected to an arterial pressure transducer (Hewlett Packard, 1280 B) and blood pressure module (H.P. 78205 A); venous blood samples were collected hourly for lactate and pyruvate determinations. Rectal temperature was measured hourly, for 6 h, in non-anaesthetized intact and lactic acidotic rats, using a telethermometer (Yellow Spring Scientific Instruments Co., Yellow Spring, Ohio, U.S.A.).

3. Substrate and Hormone Determinations

Blood was collected in chilled heparinized tubes and an aliquot immediately deproteinized in cold 30% (w/v) perchloric acid (PCA); the remainder was centrifuged at +4° C and aliquots of plasma deproteinized in cold 7.5% (w/v) PCA. Unneutralized filtrates were immediately stored at −20° C until analysis, which for pyruvate and acetoacetate was within 3 days. Under the conditions employed, no significant decrease in the concentration of substrates could be demonstrated for this time interval, provided that the samples were thawed only once, just before assay. Assay of lactate, pyruvate, alanine, ketone bodies and glycerol was by an enzymic fluorimetric method modified from published techniques [9], using an Aminco fluoromicrophotometer (American Instruments Co., Silver Spring, Md, U.S.A.) equipped with appropriate filters. Lactate [10], pyruvate [11], 3-hydroxybutyrate [12] and acetoacetate [13] were determined in blood filtrates, alanine [14] and glycerol [15] in plasma filtrates; glucose was assayed by the glucose-oxidase method [16] after deproteinization of blood with 0.16% (w/v) uranyl-acetate (0.1 ml blood/ml). Blood pH was determined within 10 min of drawing anaerobically heparinized samples, by use of a glass microelectrode (Radiometer, type E-5021 a, Copenhagen, Denmark).

4. Hormone Assays

Blood samples intended for hormone assays were collected in aprotinin (Iniprol^R − Choay, Paris, France; 2,000 U/ml), centrifuged at +4° C and kept frozen at −20° C until assay. Glucagon and insulin were determined according to the radio-immunological method [17] with slight technical adaptations [18–19]. Purified rat insulin and glucagon were used as standards (Novo Research Institute, Copenhagen, Denmark). The glucagon assay utilised the 30 K antiserum from Prof. R. H. Unger (Dallas, Texas, U.S.A.).

5. Conversion of ^{14}C-Lactate into ^{14}C-Glucose

Normal and nephrectomized rats were used, with and without phenformin treatment. In order to reach comparable blood lactate concentrations in all rats, control animals were given IV sodium lactate, a 2 ml bolus (2 mmol) prior to the injection of ^{14}C-lactate, followed by an infusion of 6.5 ml (6.5 mmol) over one hour. The blood lactate levels in control rats were stable from the 2nd min after lactate injection and were between 7 and 9 mmol/l. The lactic acidotic rats received IV sodium bicarbonate in molar and volume amounts similar to the sodium lactate load administered to control rats, in order to reach comparable blood pH and volaemic variations. Some intact rats were given glucagon (5 μg bolus then 16 μg over one hour), in order to reach plasma glucagon concentration comparable to that of lactic acidotic rats. Finally, a total of 5 groups of animals were used: normal rats, lactate-infused; normal rats, phenformin-treated and lactate-infused; normal rats, lactate- and glucagon-infused; nephrectomized rats, lactate-infused; nephrectomized rats, phenformin-treated and bicarbonate-infused.

Under light thiopental anaesthesia, catheterization of the carotid artery was performed for blood sampling, and of the dorsal vein of the penis for infusion. One μCi of L(U)^{14}C-lactate (Amersham, Bucks, England; CFB 97, specific activity 29 mCi/mmol) was injected IV, mixed with the bolus of unlabelled sodium lactate or bicarbonate.

Blood samples (0.2 ml), collected every 10 min for 30 min, then at 60 min, were deproteinized with 2.51% $ZnSO_4$ (w/v) and 2.62% $Ba(OH)_2$ (w/v), 1 ml of each. Protein-free filtrates were immediately

frozen at −20° C. The ^{14}C-glucose contained in protein-free filtrates was separated from ^{14}C-lactate using a mixture of two ion-exchange resins: 400 mg Amberlite MB3 and 100 mg Dowex 50 WX8 [20]. Recovery of ^{14}C-glucose added to blood before precipitation was 80%, whereas the recovery of added ^{14}C-lactate was less than 0.05%. An aliquot of the protein-free supernatant, before and after ion-exchange resin treatment, was counted in 10 ml of Bray's solution [21] using a scintillation spectrophotometer (Nuclear Chicago Mark I); external standards were used to correct for quenching.

Lactate specific radioactivity (dpm/μmol) was calculated for all times of sampling. The percent of administered radioactivity incorporated into glucose was calculated on the basis of an assumed "glucose space" of 30% body weight:

$$\text{Percent to glucose} = 100 \times \frac{\text{Glucose space (ml)} \times {}^{14}\text{C-glucose in blood (dpm/ml)}}{\text{Administered radioactivity (dpm)}}$$

Total production of glucose per 100 g body wt/h was calculated on the basis of an assumed "lactate space" of 60% of body wt:

$$\text{Lactate space (ml)} \times \frac{\text{Percent administered RA converted to glucose}}{2} \times \frac{100}{\text{body wt (g)}}$$

Results

1. Renal Function

Subtotal nephrectomy caused marked alteration of renal function, with serum creatinine 4.5 ± 0.1 mg/100 ml on the 1st post-operative day and 2.8 ± 0.1 mg/100 ml on the 8th day.

2. Metabolic Changes Following Phenformin Administration to Normal and Partially Nephrectomized Rats

In normal rats, phenformin administration for 8 days induced a slight but significant rise of blood lactate: from 1.07 ± 0.04 to 1.50 ± 0.03 mmol/l, and alanine: from 0.36 ± 0.02 mmol/l to 0.48 ± 0.04 ($p < 0.01$). Blood pyruvate (0.10 ± 0.01 mmol/l), plasma glycerol (0.14 ± 0.02 mmol/l) and pH (7.34 ± 0.02) did not change significantly; 3-hydroxybutyrate rose significantly from 0.21 ± 0.03 to 0.32 ± 0.03 mmol/l ($p < 0.025$) and acetoacetate from 0.12 ± 0.00 to 0.17 ± 0.01 mmol/l ($p < 0.005$). The L/P and 3-hydroxybutyrate/acetoacetate ratios did not vary significantly and remained in a range of 11 to 12 and 1.8 to 2.8 respectively. Blood glucose, after a 12 hours fasting period, was 70 ± 2 mg/100 ml; plasma insulin: 30 ± 10 μU/ml and glucagon: 163 ± 7 pg/ml, the insulin/glucagon molar ratio being in the normal range.

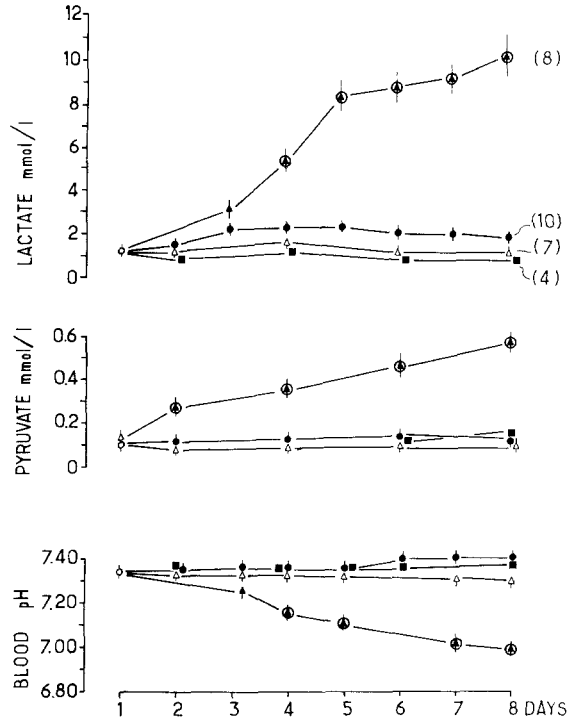

Fig. 1. Daily changes in blood lactate, pyruvate and pH, in normal rats with (●) and without (○) phenformin treatment, nephrectomized rats with (▲) and without (△) phenformin treatment and nephrectomized rats treated with hydroxy-phenformin (■). Results are presented as mean ± SEM. Circles (○) denote a significant difference with the corresponding value in phenformin-treated control rats, ($p < 0.001$). Number of experiments are between parentheses

All rats with renal failure (serum creatinine > 1.4 mg/100 ml) developed a lactic acidosis when phenformin was given. On the 8th day, blood lactate was 10.8 ± 1.0 mmol/l; pyruvate 0.56 ± 0.06 mmol/l and blood pH 7.00 ± 0.02 (Fig. 1). The L/P ratio was increased: 18.6 ± 3.4. Acetoacetate (0.51 ± 0.10 mmol/l), 3-hydroxybutyrate (1.41 ± 0.37 mmol/l) and glycerol (0.63 ± 0.07 mmol/l were also increased (Fig. 2) and the 3-hydroxybutyrate/acetoacetate ratio was increased: 3.0 ± 0.3. Alanine was extremely elevated: 1.66 ± 0.26 mmol/l. Blood glucose, after a 12 h fast, was 23 ± 8 mg/100 ml, and some rats developed convulsions. Plasma glucagon was extremely elevated: 2030 ± 170 pg/ml; plasma insulin was 23 ± 9 μU/ml and the insulin/glucagon molar ratio declined to 0.19 (Table 1).

Lactic acidosis developed earlier in the rats with severe renal failure. Out of 30 nephrectomized rats treated with phenformin, which all developed lactic acidosis, 7 had a serum creatinine level between 4.3

Table 1. Circulating substrate and hormone concentrations in the rats used for evaluation of gluconeogenesis

Group	Number of experiments	Min of lactate infusion	Glucose mg/100ml	Lactate mmol/l	Alanine mmol/l	Glucagon pg/ml	Insulin μU/ml
Normal rats	(8)	0	68±2	0.6 ±0.1	0.36±0.02	108±10	32±8
+ Lactate		60	113±3	8.6 ±1.0	0.94±0.15	60±20	45±11
Normal rats							
+ Phenformin	(8)	0	70±2	1.1 ±0.1[a]	0.48±0.04[b]	163±7[c]	30±10
+ Lactate		60	79±14[a]	9.2 ±0.6	1.20±0.09	353±29[c]	25±15
Nephrectomized rats	(5)	0	82±6	0.8 ±0.2	0.40±0.03	125±30	16±2
+ Lactate		60	117±3	8.5 ±1.6	0.96±0.02	180±60	25±15
Nephrectomized rats	(8)	0	23±8[c]	7.0 ±0.5[c]	1.66±0.26	2030±170[c]	23±9
+ Phenformin		60	7±2[c]	6.6 ±0.8	1.88±0.25	1960±196[c]	33±10
Normal rats + Lactate	(6)	0	68±2	0.64±0.03	0.36±0.02	110±20	25±10
+ Glucagon		60	110±9	7.7 ±1.1	0.44±0.02	3600±200[c]	150±25[b]

Rats, fasted for 12 h, were studied on the 8th day of treatment. For details of different groups, see "Methods". Blood lactate in the nephrectomized rats treated with phenformin was, in this study, slightly lower (7.0 ± 0.5 mmol/l) than in the group of rats used for daily follow-up (10.8 ± 1.0)

Signs denote a significant difference with the corresponding value in the lactate infused normal rats:
[a] for $p < 0.05$; [b] for $p < 0.01$ and [c] for $p < 0.001$

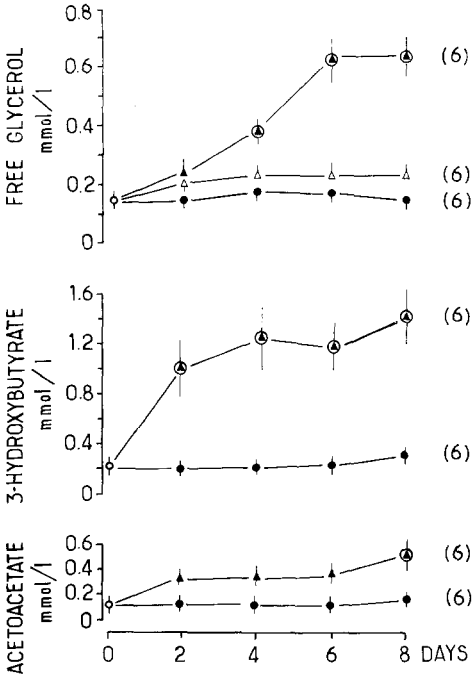

Fig. 2. Daily changes in blood 3-hydroxybutyrate, acetoacetate and free glycerol in normal rats with (●) and without (○) phenformin-treatment, nephrectomized rats with (▲) and without (△) phenformin treatment. Results are presented as in Figure 1

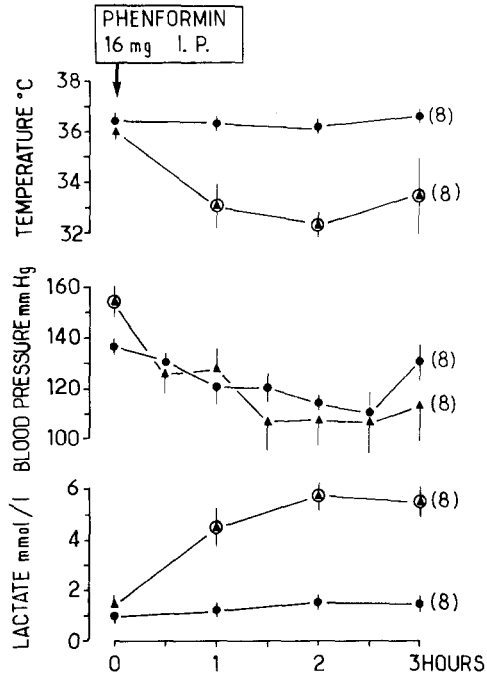

Fig. 3. Blood lactate, blood pressure and body temperature following phenformin administration. Experiments were done in intact (●) and nephrectomized (▲) rats, on the 5th day of treatment with phenformin. Results are presented as in the preceding figures

and 11.6 mg/100 ml (mean 6.9 ± 1.0): lactic acidosis (mean lactate 9 mmol/l) appeared within 3 days; the other 23 rats, with a serum creatinine level between 1.9 and 3.7 mg/100 ml (mean 2.5 ± 0.1) developed lactic acidosis (mean lactate 8.3 mmol/l) after 6 days. Lactic acidosis never appeared in the rats with a serum creatinine level below 1.4 mg/100 ml.

No significant change occurred in the nephrectomized rats injected with saline physiologic solution or with hydroxyphenformin (Figs. 1 and 2).

3. Relation between Metabolic Events and Physiological Changes in Lactic Acidotic Rats

Every daily IP injection of phenformin was followed, in the nephrectomized rats, by transient physical

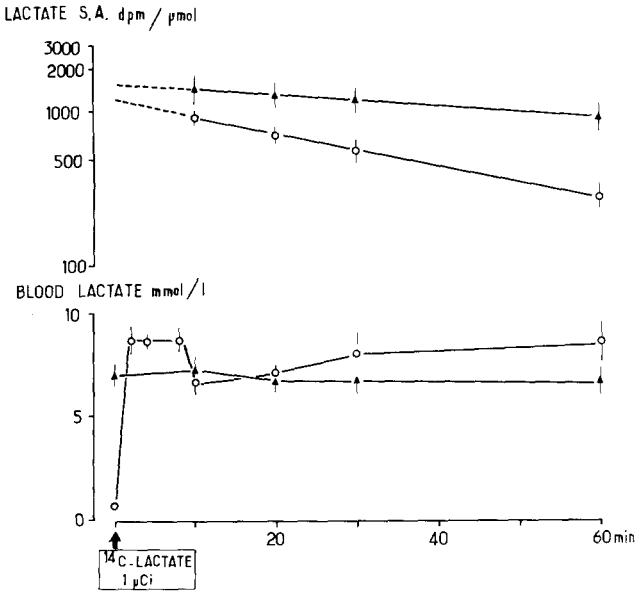

Fig. 4. Lactate specific activity (S. A.) in the lactic acidotic (▲) and control (○) rats, following the I. V. injection of 1 µCi L(U)-^{14}C-sodium lactate. Experiments were performed on the 8th day of phenformin treatment

Table 2. Incorporation of ^{14}C-lactate into glucose and glucose production from lactate

Group	Number of experiments	% ^{14}C recovered in glucose	µmol glucose formed from lactate/100 g body wt/h
Normal rats + Lactate	8	4.8 ±0.3	1046±93
Normal rats + Lactate + Phenformin	5	5.00±0.34	1346±78
Nephrectomized rats + Lactate	5	4.3 ±0.7	1057±212
Nephrectomized rats + Phenformin	8	1.88±0.17[b]	425±85[b]
Normal rats + Lactate + Glucagon	6	14.5 ±2.7[a]	3363±654[a]

Rats were fasted for 12 hours, on the 8th day of phenformin treatment. Experiments were started 2 hours after the last phenformin IP injection. For details of different groups, see "Methods"
The results presented are those measured 30 min after injection of ^{14}C-lactate
The signs denote the significance of differences versus the lactate infused controls:
[a] for $p < 0.01$ and [b] for $p < 0.001$

changes: hypothermia, coma, constriction of ear-vessels; the rats looked normal again after a few hours. These transient alterations became more and more severe with the progression of treatment; some rats in this group died 2 to 4 hours after injection before the 8th day. Blood pressure, lactate and pyruvate were measured before and for the 3 hours following phenformin injection, on the 6th day (Fig. 3). Blood pressure was significantly higher in the nephrectomized rats, before injection. It then fell in both groups, but remained above 11 mmHg in all rats. Blood lactate rose significantly from the 1st hour following injection in the lactic acidotic group, but not in the intact phenformin-treated rats; the L/P ratio did not vary significantly in either group, remaining between 12 and 14. A deep hypothermia occurred in the lactic acidotic rats: these rats did not return to a normal body temperature before the 6th hour following phenfomin injection. A strong negative correlation appeared between body temperature and blood lactate.

4. Incorporation of ^{14}C-Lactate into Glucose

These experiments were performed on the 8th day of treatment.

All normal animals, not treated with phenformin, had normal concentrations of glucose, lactate, and alanine in blood, when the incorporation experiments started (Table 1). Infusion of sodium lactate produced blood lactate concentrations equivalent to those measured in the lactic acidotic rats. A significant rise in blood glucose and alanine occurred in these lactate-infused normal rats ($p < 0.001$ in both cases).

Treatment with phenformin for 8 days induced, in the non-nephrectomized animals, a significant rise in blood lactate, alanine, and plasma glucagon, at the beginning of experiments. Then IV lactate infusion induced a significantly higher rise in blood alanine than in untreated, lactate-infused rats.

In the nephrectomized rats, not treated with phenformin, infusion of sodium lactate induced changes in blood glucose, alanine and lactate very similar to those observed in normal rats not treated with phenformin. The nephrectomized rats, phenformin-treated, displayed strikingly low blood glucose levels, associated with high lactate, alanine and plasma glucagon concentrations throughout the whole duration of experiments.

The specific activity of circulating lactate was very similar, 10 min after ^{14}C-lactate injection, in the lactate-infused normal rats and in the nephrectomized phenformin-treated rats. However, it decreased more slowly in this latter group than in control rats (Fig. 4).

Incorporation of ^{14}C lactate into glucose was significantly lower ($p < 0.001$) in the nephrectomized phenformin-treated rats than in all other groups (Table 2). In the absence of phenformin-treatment, the nephrectomized rats displayed no reductions in glucose production from lactate. Phenformin treat-

ment in normal rats did not influence significantly the incorporation of ^{14}C lactate into glucose.

Exogenous glucagon enhanced markedly glucose production from lactate in the normal rats infused with sodium lactate (Table 2).

Discussion

A reproducible model of lactic acidosis was obtained in non diabetic rats, by the combination of experimental renal failure and treatment with phenformin. A pharmacological dosage of phenformin was necessary to induce lactic acidosis within one week, presumably because the rat metabolizes phenformin at a much higher rate than the guinea-pig and man [7, 22]; furthermore, these experiments show that neither nephrectomy alone, nor phenformin-treatment by itself could have induced this metabolic syndrome. The pattern of this lactic acidosis was very similar to that observed in diabetic patients treated with biguanides, including high lactate, ketone and alanine levels, increased L/P and 3-hydroxybutyrate/acetoacetate ratios, and low glucose concentrations, in spite of high glucagon and low insulin concentrations [2, 23–25]. These similarities suggest that this experimental lactic acidosis may have many metabolic processes in common with the human syndrome. Similar patterns were observed in other animal models for lactic acidosis [26, 27].

Many features suggest an impairment of gluconeogenesis as major contributor to the increase in blood lactate. Incorporation of ^{14}C-lactate into glucose was decreased by comparison with all control animals, and particularly those which had been infused with lactate and glucagon. Furthermore, in the normal rats treated with phenformin, the hyperglycaemic effect of IV sodium lactate infusion was no longer observed, in spite of slightly raised plasma glucagon levels. Inhibition of gluconeogenesis by phenformin has been widely documented in vitro [28–31]. The range of concentration effective in vitro was 10^{-3} to 10^{-5} mol/l, i.e. that measured in the liver of lactic acidotic rats [3]. The predominant role of the kidney in excreting phenformin is well known [3–7] and accumulation of phenformin did occur in the lactic acidotic rats [3]; a similar accumulation had been suggested from assay in patients with a biguanide-induced lactic acidosis [1–3, 32, 33].

Impairment of gluconeogenesis and the role of biguanide accumulation, although presumably predominant, may not be exclusive factors. Acidosis induces large changes in organ blood flow, especially the kidney [34], which is an important site of gluconeogenesis; acidosis also decreases lactate utilization by the liver. Whether IV sodium bicarbonate corrected these changes was not verified.

The specific activity of lactate after injection of ^{14}C-lactate decreased more slowly in the lactic acidotic rats than in the lactate-infused control animals, indicating a decreased utilization of lactate rather than an increased production. An increased lactate production because of early shock following injection of phenformin seems improbable, since blood lactate was already higher than 4 mmol/l at a time when mean blood pressure was still in the range of 120–130 mmHg. But relative hypotension and deep hypothermia may have favoured an increased lactate production, according to a self-aggravated process, as suggested in some human patients with a biguanide-induced lactic acidosis [2, 32].

It must he kept in mind, finally, that quantitative interpretations of the results obtained using ^{14}C-lactate are fraught with difficulties: 1) The sizes of glucose and lactate pools were assumed, not actually measured; the presence of acidosis itself may alter lactate distribution. 2) An oxaloacetate pool is common to both the oxidative pathway of the tricarboxylic cycle and the gluconeogenic pathway [35, 36]: this renders calculations from isotope results not fully valid about the net fate of the labelled metabolite.

Other metabolic disturbances, however, are consistent with the concept of a deeply impaired gluconeogenesis. High concentrations of alanine were present in all rats with an increased lactataemia, as has been observed in lactic acidotic patients [23, 24]. The rise in alanine, following high lactate concentration in blood, was greatest in phenformin-treated rats and lowest in the glucagon-infused control rats, suggesting that synthesis of alanine was, for this C_3 substrate, a major metabolic alternative to the synthesis of glucose, or more likely, alanine uptake by the liver was inhibited to as great an extent as uptake of lactate [24].

The increased glycerol concentration may be explained by the impairment of gluconeogenesis and/or increased lipolysis. The ketoacidosis with selective rise of 3-hydroxybutyrate, also typical of the biguanide-induced lactic or metabolic [2, 3, 23–26, 32] acidosis, can hardly be explained solely by an increase in lipolysis or by the high glucagon levels: phenformin stimulates ketogenesis in the perfused rat liver in vitro [30] and causes hyperketonaemia in man [37].

The numerous similarities between the rat syndrome and the human biguanide-induced lactic acidosis suggest that the rat model may be applied to further metabolic and therapeutic investigations related to human lactic acidosis.

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References

1. Bengtsson, K., Karlberg, B., Lindgren, S.: Lactic acidosis in phenformin-treated diabetics. Acta Med. Scand. **191**, 203–208 (1972)
2. Assan, R., Heuclin, Chr., Girard, J.R., Lemaire, F., Attail, J.R.: Phenformin-induced lactic acidosis in diabetic patients. Diabetes **24**, 791–800 (1975)
3. Heuclin, Chr., Attali, J.R., Girard, J.R., Assan, R.: Hyperphenforminaemia, renal failure, lactic acidosis: human clinical condition and experimental model in rats. Diabetologia **10**, 369 (1974)
4. Wick, A.N., Stewart, C.J., Serif, G.S.: Tissue distribution of ^{14}C-labelled beta-phenethylbiguanide. Diabetes **9**, 163–165 (1960)
5. Hall, H., Rachamander, G., Glassman, J.M.: Tissue distribution and excretion of phenformin in normal and diabetic animals. Ann. N.Y. Acad. Sci. **148**, 601–611 (1968)
6. Beckmann, R.: The fate of biguanides in man. Ann. N.Y. Acad. Sci. **148**, 820–832 (1968)
7. Murphy, P.J., Wick, A.N.: Metabolism of beta-phenethylbiguanide. J. Pharm. Sci. **57**, 1125–1127 (1968)
8. Hamburger, J., Donovski, L., Marche, Cl.: Sur une technique de préparation d'une anse intestinale chez le rat pour l'étude de la dialyse du jéjunum comme traitement de l'urémie. Pathol. Biol. (Paris) **18**, 403–406 (1970)
9. Girard, J.R., Cuendet, G.J., Marliss, E.B., Kervran, A., Rieutort, M., Assan, R.: Fuels, hormones and liver metabolism at term and during the early post-natal period in the rat. J. Clin. Invest. **52**, 3190–3200 (1973)
10. Passoneau, J.V.: Lactate. In: H.U. Bergmeyer (Ed.): Methoden der Enzymatischen Analyse, 1430. Weinheim: Verlag-Chemie 1970
11. Passoneau, J.V., Lowry, O.H.: Pyruvate. In: H.U. Bergmeyer (Ed.): Methoden der Enzymatischen Analyse, 1412. Weinheim: Verlag-Chemie 1970
12. Mellanby, J., Williamson, D.H.: Beta-hydroxybutyrate. In: H.U. Bergmeyer (Ed.): Methoden der Enzymatischen Analyse, 1772. Weinheim: Verlag-Chemie 1970
13. Mellanby, J., Williamson, D.H.: Acetoacetate. In: H.U. Bergmeyer (Ed.): Methoden der Enzymatischen Analyse, 1776. Weinheim: Verlag-Chemie 1970
14. Williamson, D.H.: Alanine. In: H.U. Bergmeyer (Ed.): Methoden der Enzymatischen Analyse, 1634. Weinheim: Verlag-Chemie 1970
15. Wieland, O.: Glycerol. In: H.U. Bergmeyer (Ed.): Methoden der Enzymatischen Analyse, 1367. Weinheim: Verlag-Chemie 1970
16. Huggett, A.S.G., Nixon, D.A.: Use of glucose-oxidase, peroxidase and O-dianisidine in the determination of blood and urine glucose. Lancet **1957 II**, 368–370
17. Yalow, R.S., Berson, S.A.: Immunologic aspects of insulin. Am. J. Med. **31**, 882–891 (1961)
18. Assan, R., Tchobroutsky, G., Derot, M.: Glucagon radioimmunoassay: technical problems and recent data. Horm. Metab. Res. **5** (suppl. 1) 82–90 (1971)
19. Rosselin, G., Assan, R., Yalow, R.S., Berson, S.A.: Separation of antibody bound and unbound peptide hormones labelled with iodine 131 by talcum powder and precipitated silica. Nature **212**, 355–357 (1966)
20. Exton, J.H., Park, C.R.: Control of gluconeogenesis in liver. 1) General features of gluconeogenesis in the perfused livers of rats. J. Biol. Chem. **242**, 2622–2636 (1967)
21. Bray, G.A.: A simple, efficient liquid scintillation for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. **1**, 279–285 (1960)
22. Cook, D.E., Blair, J.B., Gilfillan, C., Lardy, H.A.: Mode of action of hypoglycemic agents. IV) Control of the hypoglycemic activity of phenylethylbiguanide in rats and guinea-pigs. Biochem. Pharmacol. **22**, 2121–2128 (1973)
23. Marliss, E.B., Aoki, T.T., Toews, C.J., Felig, P., Connon, J.J., Kyner, J., Huckabee, W.E., Cahill, G.F., Jr.: Aminoacid metabolism in lactic acidosis. Am. J. Med. **52**, 474–481 (1972)
24. Ball, S., Woods, H.F., Alberti, K.G.M.M.: Lactic acidosis, keto-acidosis and hyperalaninaemia in a phenformin-treated diabetic patient. Br. Med. J. **1974 IV**, 699–700
25. Wise, P.J., Chapman, M., Thomas, T.W., Clarkson, A.R., Harding, P.E., Edwards, J.B.: Phenformin and lactic acidosis. Br. Med. J. **1976 I**, 70–72
26. Holloway, P.H., Alberti, K.G.M.M.: Phenformin-induced lactic acidosis: prevention by dichloro-acetate. Clin. Sci. Mol. Med. **50**, 33 (1976)
27. Arieff, A.I., Guisado, R., Lazarowitz, V.C., Leach, W.: Phenformin and lactic acidosis: pathophysiology in an experimental model. Clin. Res. **83**, 1361 (1976)
28. Meyer, R., Ipaktchi, M., Clauser, H.: Specific inhibition of gluconeogenesis by biguanides. Nature **213**, 203–204 (1967)
29. Häckel, R., Häckel, H.: Inhibition of gluconeogenesis from lactate by phenylethylbiguanide in the perfused guinea-pig liver. Diabetologia **8**, 117–124 (1972)
30. Toews, C.J., Kyner, J.L., Connon, J.J., Cahill, G.F., Jr.: The effects of phenformin on gluconeogenesis in isolated perfused rat liver. Diabetes **19**, 368 (1970)
31. Cook, D.E., Blair, J.B., Lardy, H.A.: Mode of action of hypoglycemic agents. V) Studies with phenethyl-biguanide in isolated perfused rat liver. J. Biol. Chem. **248**, 5272–5277 (1973)
32. Assan, R., Heuclin, Chr., Ganeval, D., Bismuth, C., George, J., Girard, J.R.: Metformin-induced lactic acidosis in the presence of acute renal failure. Diabetologia **13**, 211–218 (1977)
33. Cowley, L.A., Karam, J.H., Matin, S.D., Löwensein, J.E.: Serum phenformin concentrations in patients with phenformin-associated lactic acidosis. Diabetes **26**, 628–631 (1977)
34. Yudkin, J., Cohen, R.D., Slack, B.: The haemodynamic effect of metabolic acidosis in the rat. Clin. Sci. Mol. Med. **50**, 177–184 (1976)
35. Krebs, H.A., Hems, R., Weideman, M.J., Speaker, N.: The fate of isotopic carbon in kidney cortex synthetizing glucose from lactate. Biochem. J. **101**, 242–246 (1966)
36. Cohen, R.D., Woods, H.F.: Clinical and biochemical aspects of lactic acidosis, pp. 234–236. Oxford: Blackwell Scientific Publications 1976
37. Nattrass, M., Todd, P.G., Hinks, L., Lloyd, B., Alberti, K.G.M.M.: Comparative effects of phenformin, metformin and glibenclamide on metabolic rhythms in maturity-onset diabetics. Diabetologia **13**, 145–152 (1977)

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