Metabolic Interactions of Dichloroacetate and Insulin in Experimental Diabetic Ketoacidosis

STUDIES ON WHOLE ANIMALS AND AFTER FUNCTIONAL HEPATECTOMY

By PERRY J. BLACKSHEAR, PAUL A. H. HOLLOWAY and K. GEORGE M. M. ALBERTI*
Nuffield Department of Medicine, Radcliffe Infirmary, Oxford OX2 6HE, U.K., and Faculty of Medicine, Chemical
Pathology, Southampton General Hospital, Tremona Road, Southampton SO9 4XY, U.K.

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1. The infusion of sodium dichloroacetate into rats with severe diabetic ketoacidosis over 4h caused a 2mm decrease in blood glucose, and small falls in blood lactate and pyruvate concentrations. Similar findings had been reported in normal rats (Blackshear *et al.*, 1974). In contrast there was a marked decrease in blood ketone-body concentration in the diabetic ketoacidotic rats after dichloroacetate treatment. 2. The infusion of insulin alone rapidly decreased blood glucose and ketone bodies, but caused an increase in blood lactate and pyruvate. 3. Dichloroacetate did not affect the response to insulin of blood glucose and ketone bodies, but abolished the increase of lactate and pyruvate seen after insulin infusion. 4. Neither insulin nor dichloroacetate stimulated glucose disappearance after functional hepatectomy, but both agents decreased the accumulation in blood of lactate, pyruvate and alanine. 5. Dichloroacetate inhibited 3-hydroxybutyrate uptake by the extra-splanchnic tissues; insulin reversed this effect. Ketone-body production must have decreased, as hepatic ketone-body content was unchanged by dichloroacetate yet blood concentrations decreased. 6. It was concluded that: (a) dichloroacetate had qualitatively similar effects on glucose metabolism in severely ketotic rats to those observed in non-diabetic starved animals; (b) insulin and dichloroacetate, both separately and together, decreased the net release of lactate, pyruvate and alanine from the extra-splanchnic tissues, possibly through a similar mechanism; (c) insulin reversed the inhibition of 3-hydroxybutyrate uptake caused by dichloroacetate; (d) dichloroacetate inhibited ketone-body production in severe ketoacidosis.

In the normal rat, sodium dichloroacetate causes a decrease in blood glucose in the 24h-starved animal (Blackshear *et al.*, 1974) but not in the fed state (Lorini & Ciman, 1962; Stacpoole & Felts, 1970, 1971). In fed alloxan-diabetic rats, Stacpoole & Felts (1970) noted hypoglycaemic effects and also found increased oxidation of [U-¹⁴C]glucose in muscle from the diabetic animals. The mechanism of action of dichloroacetate has been elucidated by Randle and his co-workers (Whitehouse & Randle, 1973; McAllister *et al.*, 1973; Whitehouse *et al.*, 1974), who have shown increased oxidation of glucose and pyruvate in muscle from normal and diabetic rats *in vitro*, and activation of pyruvate dehydrogenase (EC 1.2.4.1) in rat heart, diaphragm, adipose tissue, kidney and liver.

We have noted that insulin treatment of hyperglycaemic ketoacidotic man (Hockaday & Alberti,

1972) and rats (P. J. Blackshear & K. G. M. M. Alberti, unpublished work) results in a sharp increase in blood lactate and pyruvate concentration. We have therefore examined the metabolic effects and interactions of dichloroacetate and insulin in streptozotocin-diabetic ketoacidotic rats.

Experimental

Animals

Male rats of the Ash-Wistar strain weighing 185–215 g were used in all experiments. They were allowed free access to water and a standard laboratory rat diet (Diet 41B; Oxoid Ltd., London E1 9HF, U.K.) at all times except where stated in the text.

Experimental design

The induction of diabetes, maintenance of the animals, patterns of weight gain and the biochemical characteristics of this type of diabetic ketoacidosis in the rat have been described in detail (Blackshear & Alberti, 1974, 1975). Briefly, the animals were made

* To whom reprint requests should be addressed, at Faculty of Medicine, Chemical Pathology, Southampton General Hospital, Tremona Road, Southampton SO9 4XY, U.K.

diabetic by the intravenous injection of 150mg of streptozotocin/kg. They were maintained on daily injections of protamine-zinc insulin (3–6 i.u./day) for 7 days, with the dose based on change in body weight. At 24h after the last insulin injection body weight and most metabolic parameters measured were equivalent to those of placebo-injected control animals (Blackshear & Alberti, 1974). At 72h, pentobarbitone (60mg/kg) was administered intraperitoneally and polythene cannulae (no. 1619R, Bardic I-Catheter, C. R. Bard International Ltd., Clacton-on-Sea, Essex, U.K.; no. 2FG Intravenous Cannula, Portex Ltd., Hythe, Kent, U.K.) were inserted into the left femoral artery and vein. The rats were then placed in restraining cages and allowed free access to water but not food for the next 24h.

Infusions through the venous cannulae were begun the next day at approx. 90h after the last insulin injection. All solutions were infused at the rate of 1.2ml/h for 2 or 4h. The following infusates were used: NaCl, 0.9%; dichloroacetic acid, 5g/100ml (300mg/h per kg) in 0.9% NaCl; soluble insulin, 0.25 i.u./ml (1.5 i.u./h per kg) in 0.9% NaCl; and dichloroacetate plus insulin in the concentrations used when each was infused separately. Insulin and dichloroacetic acid were neutralized to pH 7.4 with NaOH.

Blood and liver sampling

(1) *Infusion studies.* Arterial blood samples (0.25ml) were drawn into heparinized syringes immediately before (in duplicate) and 15, 30, 60, 120 and 240min after the start of the infusion. A portion (0.2ml) of this blood was immediately deproteinized in 2.0ml of ice-cold 3% (v/v) HClO_4 ; these samples were prepared for enzymic analyses as described by Schein *et al.* (1971). After each infusion the animals were killed by cervical dislocation and the livers removed within 10s and freeze-clamped (Wollenberger *et al.*, 1960). The frozen tissue was prepared for enzymic analyses as described (Blackshear & Alberti, 1974) and followed the procedure of Williamson *et al.* (1967a). A portion of the frozen, pulverised tissue was shaken with 16vol. of chloroform-methanol (2:1, v/v) for triglyceride determination.

(2) *Functional hepatectomy studies.* Animals were anaesthetized with intravenous sodium pentobarbitone (60mg/kg body wt.) after 2h infusions of either NaCl or dichloroacetate. Functional hepatectomies were performed as described (Blackshear *et al.*, 1974) after an initial arterial blood sample (0.5ml) had been withdrawn and deproteinized. Immediately after functional hepatectomy the animals received 0.5ml of either NaCl (0.9%) or soluble insulin (4 i.u./ml) through the venous cannula. The insulin was injected after functional hepatectomy to avoid large changes in metabolic concentrations before the

initial blood sampling. Further arterial blood samples (0.5ml) were drawn at 10, 20 and 30min and processed as described above. The time elapsed between the end of the continuous infusions and the first blood sampling was 5–10min.

Results are expressed as means $\pm$ s.e.m.; significant differences were determined by Student's *t* test or, when multiple comparisons were made, Scheffé's (1953) *F* test.

Assays

Enzymic assays were performed for: glucose (Slein, 1963), acetoacetate and 3-hydroxybutyrate (Williamson *et al.*, 1962), glycerol (Eggstein & Kreutz, 1966), lactate (Hohorst *et al.*, 1959), pyruvate (Bücher *et al.*, 1963), glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963), ADP and AMP (Adam, 1963), glycerol phosphate (Hohorst, 1963a), phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate (Czok & Eckert, 1963), citrate (Gruber & Moellering, 1966), 2-oxoglutarate (Bergmeyer & Bernt, 1963), malate (Hohorst, 1963b), L-alanine (Williamson *et al.*, 1967b), L-glutamine (Lund, 1970), L-glutamate (Bernt & Bergmeyer, 1963) and L-aspartate (Pfleiderer, 1963). Pyruvate, 2-oxoglutarate and acetoacetate were determined immediately after neutralization of the acid extracts; all other hepatic metabolites were determined within 48h. Florisil-treated tissue extracts were used for the assay of the α -oxo acids and glucose (Williamson *et al.*, 1967a). Liver triglyceride was determined by the method of Eggstein & Kreutz (1966).

Special chemicals

The streptozotocin was a gift from Dr. W. E. Dulin, The Upjohn Co., Kalamazoo, Mich., U.S.A. Protamine-zinc insulin (40 i.u./ml) and soluble insulin (20 i.u./ml) were obtained from Burroughs Wellcome and Co., London HP4 2DY, U.K. Enzymes and coenzymes were supplied by Boehringer Corp. (London) Ltd., London W5 2TZ, U.K., except for glutaminase, supplied by Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. Florisil was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. Pentobarbitone sodium (Nembutal) was from Abbott Laboratories Ltd., Queensborough, Kent, U.K. and dichloroacetic acid from BDH Chemicals Ltd., Poole, Dorset, U.K.

Results

Infusion studies

(1) *Blood glucose.* Dichloroacetate alone caused a small but significant decrease in arterial blood glucose of 2mm from a starting value of 14mm after 2 and 4h of infusion (Fig. 1). Both insulin and insulin plus dichloroacetate produced rapid decreases in

Fig. 1. Changes in blood glucose concentration with infusions of NaCl, dichloroacetate, insulin, or insulin plus dichloroacetate

Values are means $\pm$ S.E.M. of concentration changes from zero time. Symbols and original values were as follows: ●, NaCl ($n=6$), 21 ± 2.7 mm; □, dichloroacetate ($n=7$), 14.0 ± 1.5 mm; ▲, insulin ($n=4$), 19.5 ± 1.5 mm; ○, insulin plus dichloroacetate ($n=6$), 14.4 ± 1.3 mm. When comparing values from NaCl-infused rats with those from dichloroacetate-infused rats, $P < 0.05$ at 120 min by Student's t test. For other experimental details see the text.

blood glucose after 2 and 4 h of infusions, from 19.5 ± 1.5 to 4.5 ± 1.5 mm and from 14.4 ± 1.3 to 0.7 ± 0.1 mm respectively.

(2) *Blood lactate and pyruvate.* Dichloroacetate alone infused for 4 h had no effect on blood lactate when compared with values from NaCl-infused control animals (Fig. 2), although in further experiments there was a small but significant decrease after 2 h (see Table 2). Pyruvate was significantly decreased at 60 and 120 min ($P < 0.05$) in the dichloroacetate-infused animals. The infusion of insulin resulted in rapid increases in both blood lactate and pyruvate of 1.2 and 0.08 mm respectively; the addition of dichloroacetate totally abolished this effect.

(3) *Blood ketone bodies and glycerol.* Dichloroacetate infusion caused increases in 3-hydroxybutyrate of 0.4 and 0.5 mm after 30 and 60 min of infusion respectively; 3-hydroxybutyrate then decreased by 2.4 mm after 240 min of infusion (Fig. 3a). Acetoacetate concentration began to fall earlier and was significantly decreased when compared with NaCl-infused animals at 120 and 240 min (Fig. 3b).

Both insulin and insulin plus dichloroacetate infusions resulted in rapid decreases in the blood concentrations of both ketone bodies. The differences in response between these two types of infusion were not significant.

Blood glycerol was 0.39 mm before dichloroacetate treatment and was still 0.36 mm after 4 h of infusion. Insulin infusion alone decreased glycerol by 0.17 mm from 0.37 mm, whereas insulin plus dichloroacetate caused a decrease of 0.20 mm from 0.26 mm at 240 min.

(4) *Liver metabolites.* Dichloroacetate caused significant decreases in the concentrations of most intermediates in the hepatic gluconeogenic pathway when administered to severely diabetic animals. Liver glucose was decreased from 18.0 ± 1.4 μ mol/g in the NaCl-infused animals to 10.0 ± 0.6 μ mol/g ($P < 0.01$) in animals receiving dichloroacetate. Hepatic lactate increased from 0.84 ± 0.10 to 1.92 ± 0.32 μ mol/g wet wt. ($P < 0.05$), and alanine increased from 0.35 ± 0.06 to 1.03 ± 0.17 μ mol/g, although [pyruvate] was not significantly changed. Both redox pairs, [lactate]/[pyruvate] and [3-hydroxybutyrate]/[aceto-

Fig. 2. Changes in blood concentrations of (a) lactate and (b) pyruvate with infusion of NaCl, dichloroacetate, insulin, or insulin plus dichloroacetate

Symbols are as in Fig. 1. When comparing lactate values from insulin-infused rats with those from animals infused with insulin plus dichloroacetate, $P < 0.01$ at 15, 30, 60 and 120 min, and $P < 0.05$ at 240 min by Student's t test. When comparing pyruvate values from NaCl-infused rats with dichloroacetate-infused rats, $P < 0.05$ at 60 and 120 min. When comparing pyruvate values from insulin-infused rats with those from animals infused with insulin plus dichloroacetate, $P < 0.01$ at 15, 30, 60, 120 and 240 min. Original values (mm) were: [lactate] NaCl, 0.80 ± 0.24 ; dichloroacetate, 0.97 ± 0.11 ; insulin, 0.66 ± 0.04 ; insulin plus dichloroacetate, 0.83 ± 0.10 ; original values (pyruvate) (mm) were: NaCl, 0.07 ± 0.02 ; dichloroacetate, 0.11 ± 0.01 ; insulin, 0.10 ± 0.01 ; insulin plus dichloroacetate, 0.13 ± 0.01 .

Fig. 3. Changes in concentrations of (a) 3-hydroxybutyrate and (b) acetoacetate with infusions of NaCl, dichloroacetate, insulin, or insulin plus dichloroacetate

Symbols are as in Fig. 1. When comparing 3-hydroxybutyrate values from NaCl-infused rats with dichloroacetate-infused animals, $P < 0.05$ at 240 min. When comparing acetoacetate values from these two types of infusion, $P < 0.05$ at 120 and 240 min by Student's t test. Original values [3-hydroxybutyrate] (mm) were: NaCl, 7.28 ± 0.70 ; dichloroacetate, 7.86 ± 0.45 ; insulin, 8.30 ± 0.66 ; insulin plus dichloroacetate, 5.89 ± 0.83 . Original values [acetoacetate] (mm) were: NaCl, 3.39 ± 0.47 ; dichloroacetate, 3.10 ± 0.27 ; insulin, 3.34 ± 0.57 ; insulin plus dichloroacetate, 2.73 ± 0.27 . For other experimental details see the text.

acetate] ratios increased markedly (6.6 to 10.4 and 2.2 to 4.4 respectively).

In contrast with the results for blood, liver ketone-body concentrations were unchanged after dichloroacetate administration. Dichloroacetate caused a twofold decrease in glycerol phosphate and an increase in hepatic triglyceride from 63 ± 17 to $217 \pm 33 \mu\text{mol/g}$ wet wt. ($P < 0.01$). Insulin reversed the effect of dichloroacetate on triglyceride. Adenine nucleotide concentrations after the different treatments are shown in Table 1. Dichloroacetate caused a marked and significant decrease in [ATP], [ADP] and total adenine nucleotides, similar to but greater than the changes seen in non-diabetic animals (Blackshear *et al.*, 1974). Insulin alone caused few changes and reversed the changes caused by dichloroacetate.

The infusions of insulin and insulin plus dichloroacetate resulted in six- and nine-fold decreases in hepatic glucose, to 2.8 ± 0.6 and $1.7 \pm 0.2 \mu\text{mol/g}$ respectively. These treatments also decreased total hepatic ketone-body concentrations from $9.0 \mu\text{mol/g}$ in the NaCl-infused animals to 5.7 and $3.2 \mu\text{mol/g}$ in the insulin- and insulin plus dichloroacetate-infused animals respectively. Other hepatic metabolite concentrations were changed little by these treatments.

Functional hepatectomy studies

In these experiments severely diabetic rats were infused with either NaCl or dichloroacetate for 120 min, after which time they were anaesthetized and the livers excluded from the circulation. The arterial blood metabolite concentrations immediately before functional hepatectomy are shown in Table 2. These results confirm decreases in blood [glucose], [3-hydroxybutyrate] and [acetoacetate] after dichloroacetate infusion. They also show that in these experiments dichloroacetate caused a 2.5-fold decrease in blood [alanine] and a significant decrease in blood [lactate], although [pyruvate] was not changed significantly. Blood [glycerol], [glutamate] and

Table 1. Concentrations of adenine nucleotides in livers of severely ketoacidotic rats after various infusions

Livers were removed from rats and freeze-clamped after infusions of NaCl, dichloroacetate, insulin, or insulin plus dichloroacetate. Values are expressed as means $\pm$ s.e.m.; significant differences were determined by Student's t test. * $P < 0.05$; ** $P < 0.01$ when groups B, C and D are compared with group A. † $P < 0.05$ and ‡ $P < 0.01$ when groups C and D are compared. The numbers in parentheses represent the number of animals in each group. For other experimental details see the text.

	Concn. ($\mu\text{mol/g}$)			
	A 0.9% NaCl (5)	B Dichloroacetate (6)	C Insulin (5)	D Insulin+dichloroacetate (3)
ATP	1.30 ± 0.13	$0.68 \pm 0.15^*$	1.14 ± 0.12	1.63 ± 0.58
ADP	1.42 ± 0.12	$0.68 \pm 0.15^{**}$	0.95 ± 0.07	$2.65 \pm 0.29^{\dagger\dagger}$
AMP	0.47 ± 0.05	0.65 ± 0.14	0.33 ± 0.05	$0.72 \pm 0.08^{\dagger\dagger}$
Total adenine nucleotides	3.19 ± 0.28	$2.22 \pm 0.24^*$	2.43 ± 0.17	$4.99 \pm 0.59^{\dagger\dagger}$

Table 2. Blood metabolite concentrations before functional hepatectomy in severely diabetic rats

Rats were infused with either NaCl or dichloroacetate for 120 min. They were then anaesthetized and blood samples drawn immediately before a functional hepatectomy was performed, as described in the text. Blood metabolite concentrations from the two groups are listed below as means $\pm$ S.E.M. The number of animals in each group is indicated in parentheses. Statistical comparisons were made by Student's *t* test: ***P* < 0.01 when values from dichloroacetate-infused rats and NaCl-infused animals were compared. After functional hepatectomy, each group below was divided into two subgroups, one of which was injected with 0.5 ml of NaCl and the other with 0.5 ml of insulin (4 units/ml). Results from these treatments are presented in Figs. 4–7. For other experimental details see the text.

	Concn. of metabolite (mM)	
	NaCl (11)	Dichloroacetate (10)
Glucose	15.5 $\pm$ 0.9	12.0 $\pm$ 0.8**
Lactate	0.50 $\pm$ 0.04	0.28 $\pm$ 0.02**
Pyruvate	0.04 $\pm$ 0.01	0.04 $\pm$ 0.00
3-Hydroxybutyrate	6.04 $\pm$ 0.48	3.72 $\pm$ 0.53**
Acetoacetate	3.04 $\pm$ 0.14	1.96 $\pm$ 0.18**
Glycerol	0.20 $\pm$ 0.02	0.18 $\pm$ 0.02
Alanine	0.17 $\pm$ 0.03	0.07 $\pm$ 0.01**
Glutamate	0.12 $\pm$ 0.01	0.14 $\pm$ 0.01
Glutamine	0.63 $\pm$ 0.08	0.64 $\pm$ 0.09

[glutamine] were unchanged compared with values from control rats.

(1) *Glucose*. Preinfusion of dichloroacetate did not alter the subsequent rate of glucose removal when the splanchnic bed was removed from the circulation (Fig. 4a). Somewhat surprisingly, the intravenous injection of 2 units of soluble insulin immediately after functional hepatectomy also did not increase the rate of glucose disappearance in either the NaCl- or the dichloroacetate-pretreated animals.

(2) *Glycerol*. The accumulation of glycerol after functional hepatectomy was similar in both the NaCl- and dichloroacetate-treated animals (Fig. 4b). When insulin was injected into two similar groups after functional hepatectomy, the accumulation of glycerol was stopped in both groups between 10 and 30 min after functional hepatectomy.

(3) *Lactate and pyruvate*. As shown in Fig. 5(a), blood lactate accumulation in the dichloroacetate-infused animals was decreased by 28% from that seen in the NaCl-infused controls. The injection of insulin after infusion of NaCl and functional hepatectomy decreased the accumulation of lactate by 80%, whereas insulin after infusion of dichloroacetate decreased lactate accumulation by 88%. The decreases in lactate accumulation after insulin or insulin plus dichloroacetate treatments were sig-

nificant (*P* < 0.01) at all time-points when compared with values from NaCl-treated animals.

Dichloroacetate pretreatment also decreased the accumulation of pyruvate after functional hepatectomy by 82% (Fig. 5b); these changes were significantly different from control values at 20 and 30 min (*P* < 0.05). The injection of insulin after NaCl infusion had less effect on pyruvate accumulation (64% decrease). The combination of dichloroacetate infusion and insulin injection completely prevented pyruvate accumulation in the blood of functionally

Fig. 4. Changes in blood concentrations of (a) glucose and (b) glycerol after functional hepatectomy

Rats were infused with NaCl or dichloroacetate for 120 min before functional hepatectomy at time 0 min; blood metabolite concentrations at this time are shown in Table 1. Immediately after each functional hepatectomy, the rats received an intravenous injection of either 0.5 ml of NaCl or 2 i.u. of soluble insulin in 0.5 ml of NaCl. ●, NaCl infusion, NaCl injection (*n* = 5); □, dichloroacetate infusion, NaCl injection (*n* = 6); ▲, NaCl infusion, insulin injection (*n* = 6); ○, dichloroacetate infusion, insulin injection (*n* = 4). Values are means $\pm$ S.E.M.; significant differences were determined by Scheffé's (1953) *F* test, and are included in the text. For other experimental details see the text.

Fig. 5. Changes in blood concentrations of (a) lactate and (b) pyruvate after functional hepatectomy

Symbols and other details are as in Fig. 4.

hepatectomized diabetic animals ($P < 0.01$ at 20 and 30 min when compared with control values).

(4) *Alanine, glutamate and glutamine.* Pretreatment with dichloroacetate decreased alanine accumulation after functional hepatectomy by 63% (Fig. 6a). A similar inhibition of alanine accumulation was seen after the injection of insulin in the NaCl-infused animals. Values from both treatments were significantly different from control values at 20 and 30 min ($P < 0.01$). Alanine accumulation was significantly decreased by 88% in the animals pretreated with dichloroacetate and injected with insulin ($P < 0.01$ at all time-points when compared with control values).

Blood [glutamate] did not change markedly in any of the four groups, although there was a slight accumulation of this amino acid in the blood of those animals pretreated and also injected with NaCl compared with a slight decrease in the other three groups (Fig. 6b).

Dichloroacetate pretreatment caused an increase in glutamine accumulation after functional hepatectomy that was twofold greater than that seen in control animals ($P < 0.05$ at 20 min, $P < 0.01$ at 30 min) (Fig. 6c). Insulin injected after the infusion of NaCl slightly decreased glutamine accumulation (34%), whereas the combination of dichloroacetate

infusion and insulin injection resulted in a normal rate of glutamine accumulation ($P < 0.01$ when compared with dichloroacetate infusion at 30 min).

(5) *Ketone bodies.* Dichloroacetate treatment caused a marked decrease in the peripheral uptake of 3-hydroxybutyrate after functional hepatectomy (Fig. 7a). Insulin injected after NaCl infusion resulted in a similar rate of 3-hydroxybutyrate disappearance to that seen in NaCl-infused, NaCl-injected control animals. Moreover, the injection of insulin after functional hepatectomy abolished the inhibition of 3-hydroxybutyrate uptake in the dichloroacetate-pretreated animals.

The results obtained for acetoacetate uptake were less definitive (Fig. 7b). Since blood [acetoacetate] was decreased after 120 min of dichloroacetate infusion (Table 1), concentration decreases after functional hepatectomy would appear less because the rate of acetoacetate uptake is proportional to its blood concentration (Williamson & Hems, 1970). Thus acetoacetate uptake was similar both in animals treated with dichloroacetate or with dichloroacetate

Fig. 6. Changes in blood concentrations of (a) alanine, (b) glutamate and (c) glutamine after functional hepatectomy

Symbols and other details are as in Fig. 4.

Fig. 7. Changes in blood concentrations of (a) 3-hydroxybutyrate, (b) acetoacetate and (c) the [3-hydroxybutyrate]/[acetoacetate] ratio after functional hepatectomy

Symbols and other details are as in Fig. 4.

plus insulin. However, inhibition of acetoacetate uptake after dichloroacetate pretreatment alone was suggested by the slower rate of increase in the blood [3-hydroxybutyrate]/[acetoacetate] ratio when compared with that from animals pretreated with insulin plus dichloroacetate. The results obtained after functional hepatectomy in rats treated in the various ways are summarized in Table 3.

Discussion

Effects of dichloroacetate and insulin on the metabolism of glucose and its precursors in ketoacidosis

It has been shown that the infusion of dichloroacetate causes hypoglycaemia in normal, 24h-starved rats (Blackshear *et al.*, 1974). These findings suggested that there was a decreased flux of gluconeogenic precursors to the liver, whereas McAllister *et al.* (1973) had shown that dichloroacetate caused increased oxidation of glucose and pyruvate in rat heart and rat diaphragm. Both of these findings were

commensurate with an activation of muscle pyruvate dehydrogenase (Whitehouse & Randle, 1973). This activation is probably due to inhibition of pyruvate dehydrogenase kinase (Whitehouse *et al.*, 1974). In diabetic animals Lorini & Ciman (1962) had noted the hypoglycaemic effect of dichloroacetate, which was confirmed by Stacpoole & Felts (1970, 1971), both groups attributing the hypoglycaemia to increased peripheral utilization of glucose.

The results described in the present study show no qualitative difference between the effects of dichloroacetate on glucose metabolism in starved normal and

Table 3. Effects of various forms of treatment on metabolite concentration changes after functional hepatectomy

In each case, the NaCl-pretreated animals receiving an injection of NaCl after functional hepatectomy are treated as the controls, with all changes expressed as relative to them. The numbers indicate the change in concentration in mM 30min after functional hepatectomy in control animals (given NaCl.) The percentages in parentheses indicate the relative magnitude of the change. —, No change compared with NaCl control. For other experimental details see the text.

Metabolite	Type of pretreatment NaCl	Dichloroacetate
Glucose		
No insulin	2.5mM disappearance	—
Insulin	—	—
Lactate		
No insulin	3.0mM accumulation	↓ (28%)
Insulin	↓ (80%)	↓ (88%)
Pyruvate		
No insulin	0.1 mM accumulation	↓ (82%)
Insulin	↓ (64%)	↓ (100%)
Glycerol		
No insulin	0.35mM accumulation	—
Insulin	↓ accumulation*	↓ Accumulation*
Alanine		
No insulin	0.4mM accumulation	↓ (63%)
Insulin	↓ (60%)	↓ (90%)
Glutamate		
No insulin	—	—
Insulin	—	—
Glutamine		
No insulin	0.35mM accumulation	↑ (90%)
Insulin	↓ (34%)	—
3-Hydroxybutyrate		
No insulin	1.0mM disappearance	↓ Uptake
Insulin	—	—
Acetoacetate		
No insulin	2.0mM disappearance	↓ Uptake (?)
Insulin	—	—

* After 10min lag period.

severely diabetic rats; thus blood [glucose], [lactate] and [pyruvate] all decreased in the diabetic animals as did the hepatic concentrations of most gluconeogenic intermediates. It is interesting that there was an increase in hepatic lactate and alanine, suggesting increased extraction with decreased utilization. One explanation could be decreased activity of pyruvate carboxylase as a result of either decreased acetyl-CoA (Utter *et al.*, 1964) or decreased ATP.

Dichloroacetate also caused a decrease in the accumulation of pyruvate, lactate and alanine in the blood of functionally hepatectomized diabetic animals. These findings, as in the normal animals, could be explained by the activation of extrahepatic pyruvate dehydrogenase (Whitehouse *et al.*, 1974). These results support the view expressed both by ourselves (Blackshear *et al.*, 1974) and by Whitehouse *et al.* (1974) that dichloroacetic acid causes hypoglycaemia by decreasing the net release of gluconeogenic precursors from extrahepatic tissues.

The infusion of insulin into the diabetic animals at a high concentration (0.3 unit/h) resulted in the expected rapid decrease in arterial blood [glucose]. This was modified by dichloroacetate (Fig. 1), although the rate of decrease may already have been at a maximum. Insulin alone caused a marked increase in blood [lactate] and [pyruvate], an effect that was abolished by the addition of dichloroacetate to the infusate. The elevation of blood [lactate] and [pyruvate] by insulin in diabetic patients has been described (Tolstoi *et al.*, 1924; Alberti & Hockaday, 1972). This was thought to be due either to increased glucose uptake and glycolysis with increased lactate and pyruvate production from the periphery, or to the inhibition by insulin of hepatic gluconeogenesis (Exton *et al.*, 1973).

The source of the increased blood [lactate] and [pyruvate] was sought in animals that had undergone functional hepatectomy. In this case the intravenous injection of 2 units of soluble insulin resulted in a decreased rate of accumulation of lactate, pyruvate, alanine and glycerol (Figs. 4, 5 and 6). Similar effects of insulin have been noted in starved, normal animals (P. J. Blackshear, unpublished work). Thus it seemed unlikely that an increase in extrahepatic lactate production was responsible for the elevation in blood [lactate] seen in the whole animals during insulin infusion. It should be noted, however, that the rats in one group were conscious, whereas the animals in the second group were anaesthetized.

These results and those from experiments in which insulin was injected after a sham operation (P. J. Blackshear & P. A. H. Holloway, unpublished work) suggest that the increases in [lactate] and [pyruvate] during insulin infusion were due mainly to a decrease in the hepatic uptake of these substances.

The mechanism of the inhibition by insulin of accumulation of lactate, pyruvate and alanine after

functional hepatectomy is unknown but may be due to increased muscle glycogen formation (Williamson, 1964; Lerner *et al.*, 1964). Alternatively, insulin may itself activate pyruvate dehydrogenase, as it does in adipose tissue (Jungas, 1971; Coore *et al.*, 1971; Wieland *et al.*, 1973) since this enzyme may be primarily in the inactive form in diabetes (Wieland *et al.*, 1971). Secondary effects of insulin, such as decreased blood free fatty acids and ketone-body concentrations, might also be responsible for an activation of muscle pyruvate dehydrogenase. This would fit in with the inhibition of accumulation of lactate, pyruvate and alanine that was seen after pretreatment with dichloroacetate (Figs. 5 and 6).

Dichloroacetate totally abolished the rise in blood [lactate] caused by insulin administration to the diabetic animals. This may have been due to both extrahepatic and hepatic actions. In the periphery there was decreased release of lactate and pyruvate into the circulation, whereas in the liver, as in muscle (McAllister *et al.*, 1973), dichloroacetate may have increased the extraction from the blood of both lactate and pyruvate.

Amino acid interactions with insulin and dichloroacetate

In 24-h-starved normal rats, dichloroacetate pretreatment before functional hepatectomy inhibited the net peripheral release of alanine while stimulating the net release of glutamine (Blackshear *et al.*, 1974). Similar effects of dichloroacetate infusion were noted after functional hepatectomy in severely ketoacidotic rats (Fig. 6). The injection of insulin into diabetic animals decreased the accumulation of both alanine and glutamine in the blood after functional hepatectomy. This could have been due both to decreased pyruvate availability and to inhibition of amino acid release from muscle (Felig, 1972; Pozefsky *et al.*, 1969). The injection of insulin after dichloroacetate pretreatment evoked an additive response: alanine accumulation was decreased more than with either agent alone, whereas glutamine accumulation was similar to that found in control animals (Fig. 6).

Effects of dichloroacetate and insulin on ketone-body metabolism

McAllister *et al.* (1973) showed that sodium dichloroacetate inhibited the oxidation of 3-hydroxybutyrate by rat diaphragm muscle, whereas we have shown that dichloroacetate increased blood [3-hydroxybutyrate] and [acetoacetate] in normal starved rats (Blackshear *et al.*, 1974). This was associated with a marked inhibition of ketone-body uptake. However, in the severely diabetic animals dichloroacetate infusion decreased blood ketone-body concentrations, and insulin plus dichloroacetate decreased blood ketone-body concentrations even further (Fig. 3). The functional-hepatectomy experiments

showed that in the diabetic animals dichloroacetate infusion inhibited peripheral ketone-body uptake. Thus both blood concentrations of ketone bodies and their peripheral uptake were decreased, implying that a decrease in hepatic ketone-body production in the dichloroacetate pretreated animals must have occurred. No antilipolytic effect of dichloroacetate could be demonstrated in these experiments, e.g. blood [glycerol] remained constant during the infusion of dichloroacetate (Table 1), and the accumulation of blood glycerol after functional hepatectomy was unaffected by dichloroacetate (Fig. 4b).

An alternative mechanism is suggested by the threefold increase in hepatic triglyceride content simultaneous with a twofold decrease in glycerol phosphate concentration after dichloroacetate treatment. This suggests that at least some of the incoming free fatty acids were being esterified rather than oxidized in this situation (Mayes & Felts, 1967).

Insulin completely reversed the dichloroacetate-induced inhibition of peripheral ketone-body uptake, although insulin alone did not appear to stimulate peripheral ketone-body uptake (Fig. 7), in contrast with the suggestions of Balasse & Havel (1971) and Bässler *et al.* (1972). McAllister *et al.* (1973) suggested that peripheral ketone-body metabolism was impaired by dichloroacetate through competition for CoA between activated pyruvate dehydrogenase and the enzymes of ketone-body oxidation. If this is indeed the case and insulin is also activating pyruvate dehydrogenase then it is difficult to explain the enhanced ketone-body clearance seen with the two agents together. McAllister *et al.* (1973) found increased tissue concentrations of acetyl-CoA after perfusion of rat hearts with dichloroacetate, which suggests a further possible mechanism. Because of the freely reversible nature of the enzymes involved in ketone-body utilization in muscle (Williamson & Hems, 1970), increased concentrations of acetyl-CoA would be expected to inhibit the formation of acetyl-CoA from ketone bodies and thus net ketone-body uptake. The inhibitory effect of insulin on tissue lipolysis might then explain the reversal by insulin of the dichloroacetate inhibition of ketone-body uptake. In this situation insulin would decrease the amount of acetyl-CoA derived from free fatty acids; this would in turn stimulate the conversion of incoming ketone bodies into acetyl-CoA and result in an increase in their net uptake by the extra-splanchnic tissues.

Thus dichloroacetate had no effect on extrahepatic glucose uptake and presumably caused hypoglycaemia in intact animals by decreasing the peripheral release of the gluconeogenic precursors lactate, pyruvate and alanine. These effects may have been due to activation of pyruvate dehydrogenase by dichloroacetate (Whitehouse & Randle, 1973; Whitehouse *et al.*, 1974). Dichloroacetate also increased the peri-

pheral release of glutamine while inhibiting the uptake of ketone bodies by the non-splanchnic tissues, both effects being reversed by insulin. The inhibition of peripheral ketone-body uptake combined with the decrease in blood concentration can only be explained by a fall in hepatic production. Finally, dichloroacetate infusion prevented the rise in blood [lactate] and [pyruvate] caused by insulin treatment in the diabetic animals.

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