

## The Metabolic Effects of Sodium Dichloroacetate in the Starved Rat

By PERRY J. BLACKSHEAR, PAUL A. H. HOLLOWAY  
and

K. GEORGE M. M. ALBERTI\*

*Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OK2 6HE, U.K.*

(Received 11 February 1974)

1. Sodium dichloroacetate (300 mg/kg body wt. per h) was infused in 24-h starved rats for 4 h. 2. Blood glucose decreased significantly, an effect that had previously only been noted in diabetic animals. 3. Plasma insulin concentration decreased by 63%; blood lactate and pyruvate concentrations decreased by 50 and 33%, whereas concentrations of 3-hydroxybutyrate and acetoacetate increased by 81 and 73% respectively. 4. Livers were freeze-clamped at the end of the 4 h infusion. There were significant decreases in hepatic [glucose], [glucose 6-phosphate], [2-phosphoglycerate], the [lactate]/[pyruvate] ratio, [citrate] and [malate], and also [alanine], [glutamate] and [glutamine], suggesting a diminished supply of gluconeogenic substrates. 5. Animals subjected to a functional hepatectomy at the end of 2 h infusions showed no difference in blood-glucose disappearance but a highly significant decrease in the rate of accumulation of lactate, pyruvate, glycerol and alanine, compared with control animals. Dichloroacetate decreased ketone-body clearance. 6. After functional hepatectomy an increase in glutamine accumulation appeared to compensate for the decrease in alanine accumulation. 7. It is concluded that dichloroacetate causes hypoglycaemia by decreasing the net release of gluconeogenic precursors from extrahepatic tissues while inhibiting peripheral ketone-body uptake. 8. These findings are consistent with the activation of pyruvate dehydrogenase (EC 1.2.4.1) in rat muscle by dichloroacetate previously described by Whitehouse & Randle (1973).

The hypoglycaemic action of di-isopropylammonium dichloroacetate was first observed in diabetic, but not in normal, fed rats by Lorini & Ciman (1962). Glycosuria was decreased and the respiratory quotient increased in the diabetic animals. They concluded that di-isopropylammonium dichloroacetate stimulated the peripheral utilization of glucose in the diabetic state. Stacpoole & Felts (1970, 1971) demonstrated that dichloroacetate was the active moiety, and that both its di-isopropyl and sodium salts stimulated [ $U$ - $^{14}C$ ]glucose oxidation and inhibited [ $1$ - $^{14}C$ ]oleate oxidation in hemidiaphragms from alloxan-diabetic rats. They too found no effect in normal fed animals.

More recently Randle and co-workers (McAllister *et al.*, 1973; Whitehouse & Randle, 1973) showed that sodium dichloroacetate decreased the blood concentrations of lactate and pyruvate and increased their extraction by heart muscle in normal dogs infused with Intralipid and heparin. Similar changes were observed in alloxan-diabetic dogs. They also reported dichloroacetate-induced increases in glucose and pyruvate oxidation and a decrease in lactate production in the perfused hearts of normal rats, and

attributed these effects to the activation of pyruvate dehydrogenase (EC 1.2.4.1) by dichloroacetate which they described in the perfused rat heart (Whitehouse & Randle, 1973). The effects of dichloroacetate on liver metabolism have not been investigated to date.

The aim of the present study was to investigate the general effects of dichloroacetate on blood and liver metabolites in normal 24-h starved rats before its use in the investigation of disorders of lactate and pyruvate metabolism.

### Experimental

#### Animals

Male Ash/Wistar rats weighing 185–215 g were used. They were allowed free access to water and a standard laboratory rat diet (diet 4lb, Oxoid Ltd., London SE1 9HF, U.K.) at all times except where stated in the text.

#### Experimental design

Fed rats were anaesthetized with sodium pentobarbitone (60 mg/kg body wt. intraperitoneally), and polythene catheters (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

\* To whom reprint requests should be addressed. Present address: Chemical Pathology, Southampton General Hospital, Tremona Road, Southampton SO9 4XY, U.K.

femoral artery and vein. The rats were then placed in restraining cages and allowed free access to water for the next 24 h. Infusions of NaCl or dichloroacetate were begun through the venous cannula when the animals had been deprived of food for 24 h. All animals were completely conscious during the infusions.

NaCl was used at a concentration of 9 g/litre; dichloroacetic acid was neutralized with NaOH and diluted with the NaCl to a final concentration of 50 g/litre (0.39 M). Both solutions were infused at 1.2 ml/h for 4 h. Previous studies had shown that the mean packed-cell-volume decrease after this type of infusion for 4 h was 6% (v/v) (P. J. Blackshear & K. G. M. M. Alberti, unpublished work).

#### *Blood and liver sampling*

(a) *Infusions only.* In one group of 12 animals, six received 4 h infusions of NaCl and six received dichloroacetate. Arterial blood samples (0.25 ml) were drawn into heparinized syringes at times 0 (in duplicate), 15, 30, 60, 120 and 240 min. A portion of this blood (0.2 ml) was immediately deproteinized in 2.0 ml of ice-cold 3% (v/v) HClO<sub>4</sub>; these samples were prepared for enzymic analyses as described previously (Schein *et al.*, 1971). After each infusion, the animals were killed by cervical dislocation and the livers removed within 10 s and freeze-clamped (Wollenberger *et al.*, 1960). The frozen tissue was prepared for enzymic analyses as described previously (Blackshear & Alberti, 1974) and followed the procedure of Williamson *et al.* (1967a). A portion of the frozen, pulverized tissue was shaken with 16 vol. of chloroform-methanol (2:1, v/v) for triglyceride determination.

A second group of ten rats received only 1 h infusions, five receiving NaCl and five dichloroacetate. Samples (0.7 ml) were drawn from these animals into EDTA (final concn. in blood, 5 mM) at 0, 15, 30 and 60 min. The plasma was separated from these samples and used for the determination of immunoreactive insulin and free fatty acids.

(b) *Functional hepatectomy studies.* For this, 16 further animals were cannulated in the usual manner, with eight receiving NaCl and eight dichloroacetate for 2 h. Immediately after each infusion the animals were anaesthetized with intravenous sodium pentobarbitone (60 mg/kg) and ligatures were placed around the coeliac and superior mesenteric arteries and the hepatic portal vein. In the rat, the coeliac and superior mesenteric arteries supply all of the abdominal viscera, including the liver, except the kidneys, descending colon and rectum (Greene, 1968). When the hepatic artery and portal vein are occluded, a functional hepatectomy is produced; the other vessels were occluded to prevent pooling of blood in the viscera.

In half of these animals, an arterial blood sample (0.6 ml) was drawn at 0 min, and the vessels were occluded immediately; further samples (0.3 ml) were drawn at 10, 20 and 30 min, deproteinized and processed in the usual way. In the remaining animals samples were drawn at 2, 3, 6 and 10 min after functional hepatectomy. The time elapsed between the end of the infusions and the first blood sampling was 5–10 min.

#### *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 48 h. Florisil-treated tissue extracts were used for the assay of the  $\alpha$ -oxo acids and glucose (Williamson *et al.*, 1967a). Plasma non-esterified fatty acids were determined colorimetrically (Itaya & Ui, 1965), liver triglyceride by the method of Eggstein & Kreutz (1966), and plasma immunoreactive insulin was measured by a micro-modification of the method of Soeldner & Slone (1965).

Results are expressed as means  $\pm$  S.E.M.; significant differences were determined by using Student's *t* test for paired or non-paired populations.

#### *Special chemicals*

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. Rat insulin standard was a gift from Novo Industries, Copenhagen, Denmark; <sup>125</sup>I-labelled insulin was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and antisera were from Wellcome Laboratories, Beckenham, Kent, U.K.

**Results**

*Effects of dichloroacetate infusion*

(a) *Blood.* The infusion of 300 mg of dichloroacetate/kg body wt. per h caused rapid and significant decreases in arterial [glucose], [lactate] and [pyruvate] in 24h-starved normal rats compared with NaCl-infused animals (Fig. 1). Blood [glucose] rose by approximately 1mM during the course of the 4h NaCl infusions in control animals. The reasons for this are not clear. Dichloroacetate also produced a significant decrease in plasma immunoreactive insulin concentration (0min,  $13 \pm 1.3 \mu\text{units/ml}$ ;

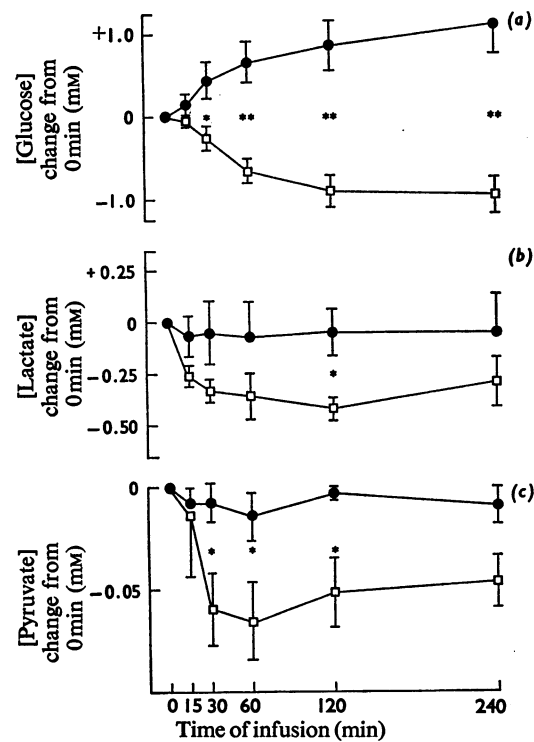


Fig. 1. Arterial blood [glucose] (a), [lactate] (b) and [pyruvate] (c) changes with infusion of NaCl or dichloroacetate

●, NaCl (n = 6); □, dichloroacetate (n = 6). Values are means ± s.e.m. of concentration changes from zero time. \* P < 0.05; \*\* P < 0.01, comparing means of the two groups at each time-point. Pre-infusion concentrations of glucose, lactate and pyruvate for the NaCl-infused rats were as follows: [glucose],  $3.73 \pm 0.19 \text{mM}$ ; [lactate],  $0.85 \pm 0.06 \text{mM}$ ; [pyruvate],  $0.08 \pm 0.01 \text{mM}$ . The values for the dichloroacetate-infused rats were: [glucose],  $3.94 \pm 0.26 \text{mM}$ ; [lactate],  $0.66 \pm 0.06 \text{mM}$ ; [pyruvate],  $0.12 \pm 0.01 \text{mM}$ . Other details are described in the Experimental section.

15min,  $9.8 \pm 2.9 \mu\text{units/ml}$ ; 30min,  $4.8 \pm 2.0 \mu\text{units/ml}$ ; 60min,  $8.5 \pm 3.0 \mu\text{units/ml}$ ; P < 0.01 when comparing 0min value with that at 30min by using paired t test). No significant changes in plasma immunoreactive insulin concentrations were observed in the animals infused with NaCl.

Dichloroacetate infusions also caused highly significant increases in arterial [3-hydroxybutyrate] and [acetoacetate], both metabolites exceeding the values for the control animals by about 1mM at 60min (Fig. 2). Blood [glycerol] remained unchanged during both control and dichloroacetate infusions. Dichloroacetate but not NaCl infusions also caused a decrease in plasma free fatty acid concentrations: 0min,  $1.04 \pm 0.03 \text{mequiv.}$ ; 15min,  $0.96 \pm 0.03 \text{mequiv.}$ ; 30min,  $0.78 \pm 0.05 \text{mequiv.}$ ; 60min,  $0.77 \pm 0.06 \text{mequiv.}$  (P < 0.01 when comparing 0min value with those at 30 and 60min by using paired t test).

(b) *Liver.* The concentrations of metabolites in the freeze-clamped livers from 24h-starved normal rats

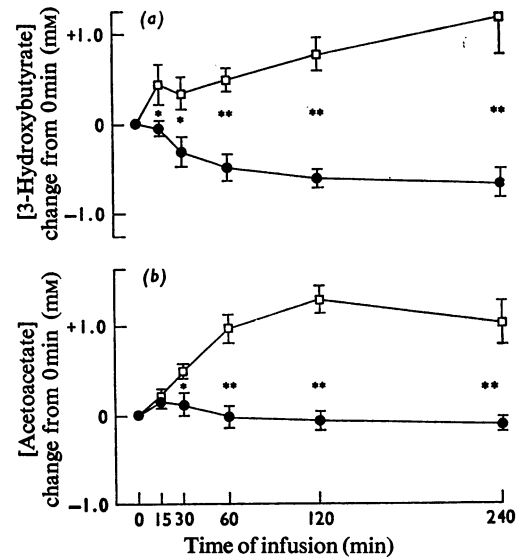


Fig. 2. Arterial blood [3-hydroxybutyrate] (a) and [acetoacetate] (b) changes with infusion of NaCl or dichloroacetate

●, NaCl (n = 6); □, dichloroacetate (n = 6). Values are means ± s.e.m. of concentration changes from zero time. \* P < 0.05; \*\* P < 0.01, comparing means of the two groups at each time-point. Pre-infusion concentrations of 3-hydroxybutyrate and acetoacetate were as follows: NaCl-infused rats: [3-hydroxybutyrate],  $1.93 \pm 0.15 \text{mM}$ ; [acetoacetate],  $1.34 \pm 0.14 \text{mM}$ ; dichloroacetate-infused rats: [3-hydroxybutyrate],  $1.52 \pm 0.26 \text{mM}$ ; [acetoacetate],  $1.38 \pm 0.18 \text{mM}$ . Other details are described in the Experimental section.

Table 1. Concentrations of metabolic intermediates in livers of normal rats after infusions with NaCl or dichloroacetate. Livers were removed from rats and freeze-clamped after 240 min of infusion with NaCl ( $n = 6$ ) or dichloroacetate ( $n = 8$ ). Values are expressed as means  $\pm$  S.E.M.; significant differences were determined by using Student's  $t$  test: \* $P < 0.05$ ; and \*\* $P < 0.01$ , when compared with animals receiving NaCl only. For other experimental details, see the text.

Metabolite ( $\mu\text{mol/g}$ )	Type of infusion	
	NaCl	Dichloroacetate
Glucose	$5.49 \pm 0.51$	$3.37 \pm 0.31^{**}$
Glucose 6-phosphate	$0.08 \pm 0.02$	$0.02 \pm 0.00^{**}$
3-Phosphoglycerate	$0.15 \pm 0.02$	$0.12 \pm 0.01$
2-Phosphoglycerate	$0.12 \pm 0.02$	$0.03 \pm 0.00^{**}$
Phosphoenolpyruvate	$0.05 \pm 0.01$	$0.03 \pm 0.00$
Pyruvate	$0.03 \pm 0.00$	$0.07 \pm 0.01^{**}$
Lactate	$0.52 \pm 0.04$	$0.46 \pm 0.04$
Citrate	$0.27 \pm 0.01$	$0.18 \pm 0.01^{**}$
2-Oxoglutarate	$0.04 \pm 0.01$	$0.06 \pm 0.01$
Malate	$0.26 \pm 0.03$	$0.19 \pm 0.01^*$
$\alpha$ -Glycerophosphate	$0.23 \pm 0.02$	$0.22 \pm 0.01$
3-Hydroxybutyrate	$2.01 \pm 0.26$	$2.42 \pm 0.20$
Acetoacetate	$0.69 \pm 0.08$	$2.20 \pm 0.28^{**}$
Triglyceride	$32.7 \pm 4.9$	$32.7 \pm 2.7$
ATP	$2.01 \pm 0.08$	$1.88 \pm 0.13$
ADP	$2.79 \pm 0.14$	$1.98 \pm 0.34$
AMP	$0.63 \pm 0.05$	$0.44 \pm 0.04^*$
Total adenine nucleotides	$5.42 \pm 0.16$	$4.30 \pm 0.44$
Glutamate	$2.10 \pm 0.15$	$1.68 \pm 0.08^*$
Glutamine	$5.21 \pm 0.66$	$3.94 \pm 0.23$
Aspartate	$0.72 \pm 0.06$	$0.40 \pm 0.05^{**}$
Alanine	$0.51 \pm 0.04$	$0.32 \pm 0.02^{**}$
[Lactate]	$18.9 \pm 2.12$	$7.20 \pm 1.25^{**}$
[Pyruvate]		
[3-Hydroxybutyrate]	$2.96 \pm 0.23$	$1.28 \pm 0.24^{**}$
[Acetoacetate]		

receiving 4h infusions of 0.9% NaCl or dichloroacetate are listed in Table 1. Dichloroacetate infusion caused highly significant decreases in the concentrations of several glycolytic intermediates, including [glucose], [glucose 6-phosphate] and [2-phosphoglycerate], as well as more than a twofold increase in [pyruvate] and a 2.5-fold decrease in the hepatic [lactate]/[pyruvate] ratio. Of the tricarboxylic acid-cycle intermediates both [citrate] and [malate] were significantly decreased in the livers from dichloroacetate-treated animals. There was no change in water content of the liver after dichloroacetate infusion (dry wt. as % wet wt.:  $35.5 \pm 1.0$  for dichloroacetate and  $35.2 \pm 0.7$  for saline-infused controls).

Of the fat metabolites, only [acetoacetate] was significantly increased in dichloroacetate-treated animals, resulting in a twofold decrease in the [3-hydroxybutyrate]/[acetoacetate] ratio. Liver triglyceride concentrations were identical in the two groups.

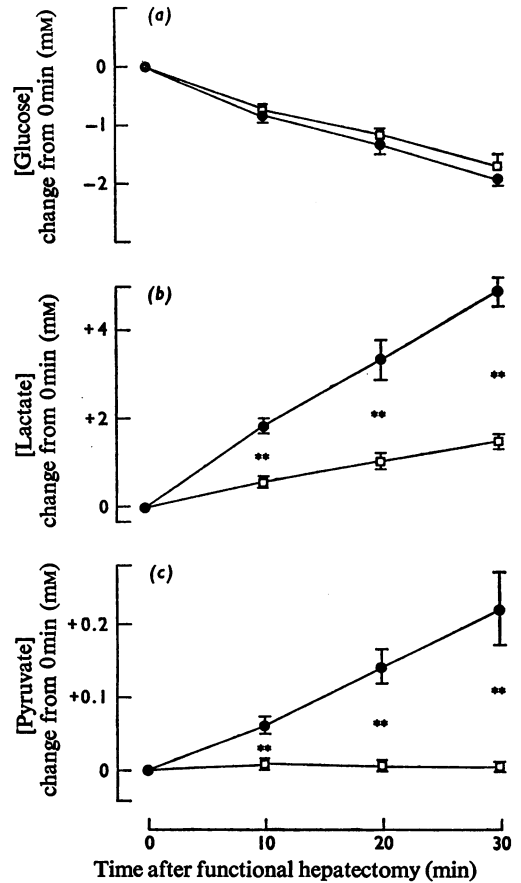


Fig. 3. Arterial blood [glucose] (a), [lactate] (b) and [pyruvate] (c) changes after functional hepatectomy

Rats were infused with NaCl ( $\bullet$ ) ( $n = 4$ ) or dichloroacetate ( $\square$ ) ( $n = 4$ ) for 2h before functional hepatectomy at zero time. Values are means  $\pm$  S.E.M. of concentration changes from zero time. \* $P < 0.05$ ; \*\* $P < 0.01$ , comparing means of the two groups at each time-point. Concentrations of blood glucose, lactate and pyruvate immediately before functional hepatectomy were as follows for NaCl-infused rats: [glucose],  $4.28 \pm 0.20$  mm; [lactate],  $0.66 \pm 0.33$  mm; [pyruvate],  $0.05 \pm 0.00$  mm; for dichloroacetate-infused rats: [glucose],  $2.63 \pm 0.06$  mm; [lactate],  $0.37 \pm 0.03$  mm; [pyruvate],  $0.02 \pm 0.00$  mm. These differences were all significant ( $P < 0.01$ ) when comparing values from NaCl-infused rats with those from dichloroacetate-infused animals. Other details are described in the Experimental section.

The [total adenine nucleotides] were slightly decreased in the dichloroacetate-treated animals, with only the decrease in [AMP] being significant ( $P < 0.05$ ). Of the amino acids measured [glutamate], [aspartate] and [alanine] were all significantly decreased in the dichloroacetate-treated animals.

*Blood metabolite concentrations in functionally hepatectomized rats*

The blood metabolite concentrations of rats after 2h infusions of either NaCl or dichloroacetate and immediately before functional hepatectomies were performed are shown in the legends for Figs. 3, 4, 5

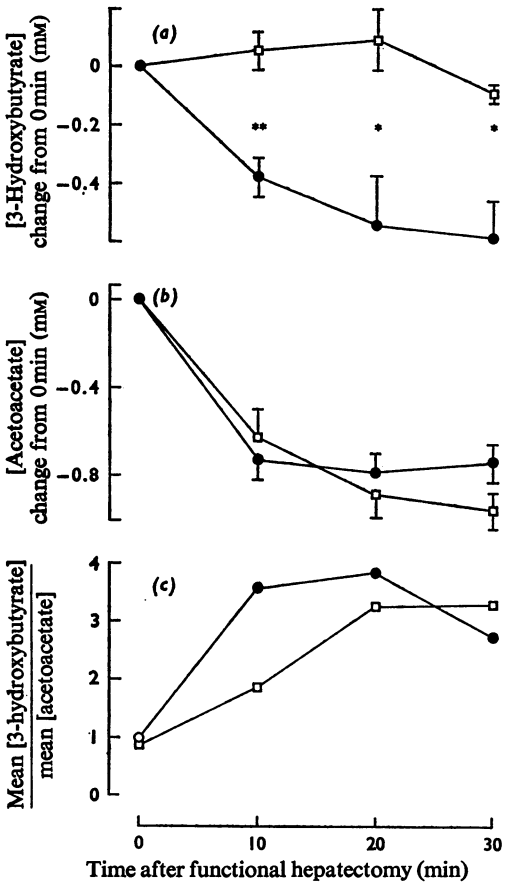


Fig. 4. Arterial blood [3-hydroxybutyrate] (a) and [acetoacetate] (b) changes and [3-hydroxybutyrate]/[acetoacetate] (c) after functional hepatectomy

Concentrations of blood 3-hydroxybutyrate and acetoacetate immediately before functional hepatectomy were as follows for NaCl-infused rats: [3-hydroxybutyrate],  $0.85 \pm 0.15$  mM; [acetoacetate],  $0.85 \pm 0.08$  mM; for dichloroacetate-infused rats: [3-hydroxybutyrate],  $1.14 \pm 0.20$  mM; [acetoacetate],  $1.26 \pm 0.15$  mM. The difference in [acetoacetate] when comparing values from NaCl-infused rats with those from dichloroacetate-infused animals was marginally significant ( $P < 0.05$ ). All other details are as in Fig. 3.

and 6. The data confirm the hypoglycaemic action of dichloroacetate in normal 24h-starved rats and also confirm its ability significantly to lower arterial [lactate] and [pyruvate].

Fig. 3 shows the concentration changes observed after functional hepatectomy for arterial [glucose],

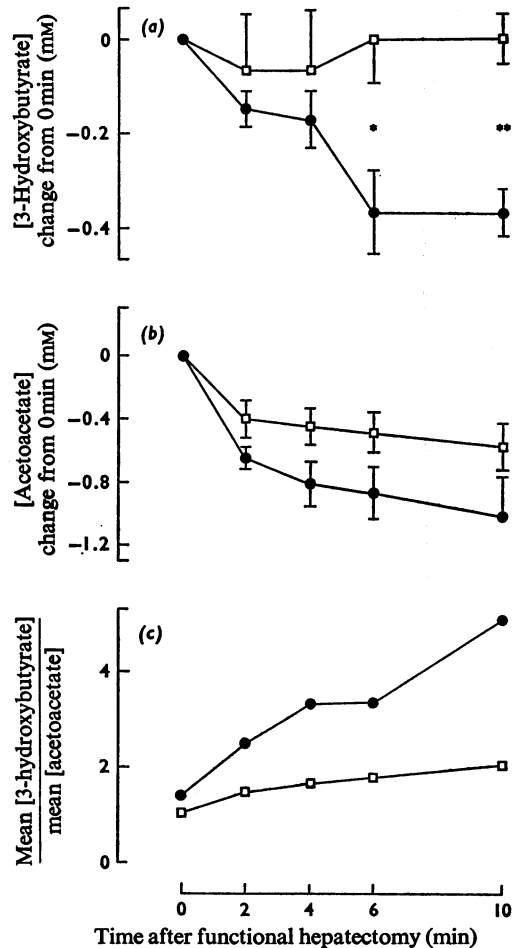


Fig. 5. Arterial blood [3-hydroxybutyrate] (a) and [acetoacetate] (b) changes, and [3-hydroxybutyrate]/[acetoacetate] (c) after functional hepatectomy measured by using rapid blood sampling

Concentrations of blood 3-hydroxybutyrate and acetoacetate immediately before functional hepatectomy were as follows for NaCl-infused rats: [3-hydroxybutyrate],  $1.84 \pm 0.63$  mM; [acetoacetate],  $1.31 \pm 0.28$  mM; dichloroacetate-infused rats: [3-hydroxybutyrate],  $1.35 \pm 0.13$  mM; [acetoacetate],  $1.24 \pm 0.14$  mM. All other details are as in Fig. 3.

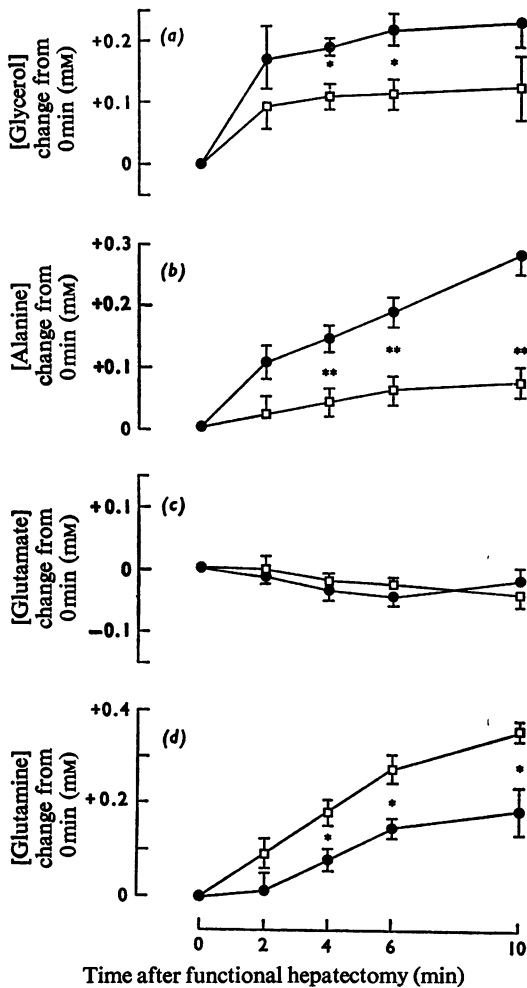


Fig. 6. Arterial blood [glycerol] (a), [alanine] (b), [glutamate] (c) and [glutamine] (d) changes after functional hepatectomy

Concentrations of blood glycerol, alanine, glutamate and glutamine immediately before functional hepatectomy were as follows for NaCl-infused rats: [glycerol],  $0.19 \pm 0.01$  mM; [alanine],  $0.12 \pm 0.03$  mM; [glutamate],  $0.18 \pm 0.01$  mM; [glutamine],  $0.45 \pm 0.03$  mM; for dichloroacetate-infused rats: [glycerol],  $0.18 \pm 0.01$  mM; [alanine],  $0.07 \pm 0.02$  mM; [glutamate],  $0.23 \pm 0.01$  mM; [glutamine],  $0.48 \pm 0.03$  mM. Only [glutamate] was significantly different in the dichloroacetate-infused animals ( $P < 0.05$ ). All other details are as in Fig. 3.

[lactate] and [pyruvate] in the two groups of animals. Blood glucose concentrations decreased in an almost identical manner after functional hepatectomy, despite the significantly different initial and final concentrations; the mean blood [glucose] of  $0.92 \pm$

0.18 mM in the dichloroacetate-pretreated animals at 30 min could possibly explain the death of all four animals between 30 and 40 min after functional hepatectomy. As shown in Figs. 3(b) and 3(c), pretreatment with dichloroacetate significantly inhibited the rapid increases in blood [lactate] and [pyruvate] seen after functional hepatectomy in the NaCl-pretreated controls; indeed [pyruvate] remained unchanged in the dichloroacetate-pretreated animals.

Fig. 4 shows the changes observed in blood [3-hydroxybutyrate] and [acetoacetate] in the two groups after functional hepatectomy. [3-Hydroxybutyrate] decreased strikingly in the control animals after functional hepatectomy (Fig. 4a), whereas little change in concentration occurred in the dichloroacetate-pretreated rats until 30 min, when a slight decrease was observed. [Acetoacetate], on the other hand, decreased rapidly and similarly in the two groups (Fig. 4b). However, decreased peripheral acetoacetate uptake was suggested by the slower increase in the [3-hydroxybutyrate]/[acetoacetate] ratio (Fig. 4c) and the proportionately smaller decrease in [acetoacetate] after functional hepatectomy in the dichloroacetate-pretreated rats.

In an attempt to characterize further this early rapid decrease in ketone-body concentrations, blood samples were drawn at shorter time-intervals after functional hepatectomy in a second group of NaCl- and dichloroacetate-pretreated rats. As in the earlier series, [3-hydroxybutyrate] decreased rather slowly in the NaCl-pretreated animals but remained constant in those pretreated with dichloroacetate; the differences were significant at 6 and 10 min (Fig. 5a). [Acetoacetate] decreased much more rapidly in both groups of animals (Fig. 5b); this decrease was apparently slower in the dichloroacetate-pretreated animals, and resulted in a much less rapid increase in the [3-hydroxybutyrate]/[acetoacetate] ratio despite the concomitantly constant [3-hydroxybutyrate] (Fig. 5c).

Rapid sampling was also used to study changes in glycerol and several amino acids (Fig. 6). Although arterial blood [glycerol] did not change significantly during dichloroacetate infusion in the whole animal, Fig. 6(a) shows that its peripheral release was significantly inhibited by pretreatment with dichloroacetate. The release of alanine from the periphery was also severely inhibited after functional hepatectomy by pretreatment with dichloroacetate. It should be noted that blood [alanine] was already decreased before hepatectomy in the dichloroacetate-treated animals (Fig. 6b). [Glutamate] did not change significantly after functional hepatectomy in either group (Fig. 6c). However, in marked contrast with [alanine], [glutamine] increased more rapidly in the dichloroacetate-pretreated animals than in the controls (Fig. 6d).

## Discussion

### *Hypoglycaemia*

Two possibilities may explain the marked decrease in blood glucose after infusion of dichloroacetate (Fig. 1): (1) increased uptake of glucose by peripheral tissues, or (2) a decrease in hepatic gluconeogenesis. As the hypoglycaemia is accompanied by a decrease in circulating lactate and pyruvate (Fig. 1) and a lowering of plasma insulin it would seem unlikely that increased uptake of glucose is the explanation. The lower concentrations of many hepatic metabolites involved in gluconeogenesis after dichloroacetate infusion (Table 1) strongly suggest that decreased gluconeogenesis is the reason for the hypoglycaemia. The fact that dichloroacetate does not decrease blood sugar in fed rats (Lorini & Ciman, 1962; Stacpoole & Felts, 1970, 1971) would support this contention. It is worth noting that the dose of dichloroacetate used in the present study is similar to those used in all previous work *in vivo* (Lorini & Ciman, 1962; Stacpoole & Felts, 1970, 1971; McAllister *et al.*, 1973); both six- and sixty-fold lower doses were without effect in our hands (P. J. Blackshear & K. G. M. M. Alberti, unpublished work). This raises the question as to the mechanism of the decrease in hepatic gluconeogenesis. Unlike the metabolite patterns observed in liver with other hypoglycaemic agents (Veneziale *et al.*, 1967; Holland *et al.*, 1973) there is no marked accumulation of any gluconeogenic intermediate which might indicate the site of action of dichloroacetate. Indeed the metabolite pattern suggests an absolute deficiency in the availability of gluconeogenic precursors (e.g. alanine, aspartate, glutamine). The increase in hepatic [pyruvate] may be due to an alteration in cytoplasmic [NAD]/[NADH] ratio; there is no change in [lactate+pyruvate].

Confirmation of the decreased availability of gluconeogenic precursors after dichloroacetate infusion comes from the experiments with functional hepatectomy. Here there was a decreased accumulation of lactate, pyruvate, glycerol and alanine (Figs. 3 and 6), while peripheral uptake of glucose remained constant (Fig. 3). The mechanism for this decreased accumulation is probably the activation of pyruvate dehydrogenase (EC 1.2.4.1) as described in perfused rat heart by Whitehouse & Randle (1973), which allows increased oxidation of lactate and pyruvate (McAllister *et al.*, 1973).

Alanine has been recognized for some time as a major carrier of amino acid-derived nitrogen from the periphery to the liver, probably from transamination of pyruvate (Mallette *et al.*, 1969; Felig *et al.*, 1970; Felig, 1973). Glutamine, which is also released in amounts disproportionately higher than its concentration in skeletal-muscle protein from the human forearm (Marliss *et al.*, 1971) and from the perfused rat hindquarter (Ruderman & Lund, 1972) may be

equally important as an amino-group carrier, although recent studies have suggested that most of this glutamine is taken up by the gut rather than the liver (Aikawa *et al.*, 1973; Matsutaka *et al.*, 1973). In our experiments, glutamine accumulation increased, after the entire splanchnic bed had been tied off in dichloroacetate-treated rats (Fig. 6), by the same amount that alanine accumulation decreased. This suggests a mechanism which can control the relative release of alanine and glutamine depending on the availability of pyruvate and amino-group donors.

### *Hyperketonaemia*

The increased [3-hydroxybutyrate] and [acetoacetate] seen during dichloroacetate infusion (Fig. 2) suggest either increased hepatic ketogenesis or decreased peripheral utilization of these substances. The decreased hepatic [3-hydroxybutyrate]/[acetoacetate] ratio implies a decrease in hepatic free fatty acid oxidation (Table 1) (Williamson *et al.*, 1971). The constant hepatic [triglyceride] and decreased plasma [free fatty acid] after dichloroacetate infusion suggest that decreased lipolysis could be responsible. Experiments with functional hepatectomy confirm a decrease in the peripheral release of glycerol (Fig. 6), and demonstrate a marked inhibition of peripheral ketone-body uptake in dichloroacetate-treated animals. The decreased uptake of 3-hydroxybutyrate confirms the finding of McAllister *et al.* (1973) that 3-hydroxybutyrate oxidation was inhibited by dichloroacetate treatment in rat muscle. The mechanism of this inhibition may be a competition for CoA between activated pyruvate dehydrogenase (Whitehouse & Randle, 1973) and the enzymes required for ketone-body utilization which also require CoA, as proposed by McAllister *et al.* (1973); a further possibility is the inhibition of 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) both in the liver and in muscle by dichloroacetate.

### *Conclusion*

The results of this study clarify the metabolic effects of dichloroacetate *in vivo*. Its hypoglycaemic action in normal starved rats appears to be due to the decreased peripheral release of the gluconeogenic substrates lactate, pyruvate, glycerol and alanine. The elevation of ketone-body concentrations in animals treated with dichloroacetate, in the face of diminished lipolysis, apparently results from decreased peripheral uptake and utilization of these substances. Finally, experiments with dichloroacetate-pretreated functionally hepatectomized animals suggest that alanine and glutamine can, to some extent, serve as carriers for a common pool of amino nitrogen from the periphery. Many of the results can be explained by increased peripheral pyruvate

oxidation; further study is required to determine the mechanism of the dichloroacetate-induced inhibition of peripheral ketone-body uptake.

We are grateful to the Rhodes Trust, the Medical Research Council and the Wellcome Trust for generous financial support. We thank Professor P. J. Randle for helpful discussions and many useful suggestions, Dr. D. H. Williamson and Professor P. B. Beeson for advice and encouragement and Mrs. H. Dhar and Mrs. S. Slade for excellent technical assistance.

## References

- Adam, H. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 573–577, Academic Press Inc., New York
- Aikawa, T., Matsutaka, H., Yamamoto, H., Okuda, T., Ishikawa, E., Kawano, T. & Matsumura, E. (1973) *J. Biochem. (Tokyo)* **74**, 1003–1007
- Bergmeyer, H.-U. & Bernt, E. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 324–327, Academic Press Inc., New York
- Bernt, E. & Bergmeyer, H.-U. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 384–388, Academic Press Inc., New York
- Blackshear, P. J. & Alberti, K. G. M. M. (1974) *Biochem. J.* **138**, 107–117
- Bücher, T., Czok, R., Lamprecht, W. & Latzko, E. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 253–259, Academic Press Inc., New York
- Czok, R. & Eckert, L. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 229–233, Academic Press Inc., New York
- Eggstein, M. & Kreutz, F. H. (1966) *Klin. Wochenschr.* **44**, 262–267
- Felig, P. (1973) *Metab. Clin. Exp.* **22**, 179–207
- Felig, P., Pozefsky, T., Marliss, E. & Cahill, G. F., Jr. (1970) *Science* **167**, 1003–1004
- Greene, E. N. (1968) *Anatomy of the Rat*, p. 199, Hafner Publishing Co., New York and London
- Gruber, W. & Moellering, H. (1966) *Biochem. Z.* **346**, 85–88
- Hohorst, H. J. (1963a) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 215–219, Academic Press Inc., New York
- Hohorst, H. J. (1963b) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 328–332, Academic Press Inc., New York
- Hohorst, H. J., Kreutz, F. H. & Bücher, T. (1959) *Biochem. Z.* **332**, 18–46
- Holland, P. C., Clark, M. G., Bloxham, D. P. & Lardy, H. A. (1973) *J. Biol. Chem.* **248**, 6050–6056
- Itaya, K. & Ui, M. (1965) *J. Lipid Res.* **6**, 16–20
- Lamprecht, W. & Trauttschold, I. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 543–551, Academic Press Inc., New York
- Lorini, M. & Ciman, M. (1962) *Biochem. Pharmacol.* **11**, 823–827
- Lund, P. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H.-U., ed.), pp. 1670–1673, Verlag Chemie, Weinheim/Bergstr.
- Mallette, L. E., Exton, J. H. & Park, C. R. (1969) *J. Biol. Chem.* **244**, 5713–5723
- Marliss, E. B., Aoki, T. T., Pozefsky, T., Most, A. S. & Cahill, G. F., Jr. (1971) *J. Clin. Invest.* **50**, 814–817
- Matsutaka, H., Aikawa, T., Yamamoto, H. & Ishikawa, E. (1973) *J. Biochem. (Tokyo)* **74**, 1019–1029
- McAllister, A., Allison, S. P. & Randle, P. J. (1973) *Biochem. J.* **134**, 1067–1081
- Pfleiderer, G. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 381–383, Academic Press Inc., New York
- Ruderman, N. B. & Lund, P. (1972) *Israel J. Med. Sci.* **8**, 295–302
- Schein, P. S., Alberti, K. G. M. M. & Williamson, D. H. (1971) *Endocrinology* **89**, 827–834
- Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 117–123, Academic Press Inc., New York
- Soeldner, J. S. & Slone, D. (1965) *Diabetes* **14**, 771–779
- Stacpoole, P. W. & Felts, J. M. (1970) *Metab. Clin. Exp.* **19**, 71–78
- Stacpoole, P. W. & Felts, J. M. (1971) *Metab. Clin. Exp.* **20**, 830–834
- Veneziale, C. M., Walter, P., Kneer, N., & Lardy, H. A. (1967) *Biochemistry* **6**, 2129–2138
- Whitehouse, S. & Randle, P. J. (1973) *Biochem. J.* **134**, 651–653
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
- Williamson, D. H., Lund, P. & Krebs, H. A. (1967a) *Biochem. J.* **103**, 514–527
- Williamson, D. H., Lopes-Vieira, O. & Walker, B. (1967b) *Biochem. J.* **104**, 497–502
- Williamson, D. H., Mayor, F. & Veloso, D. (1971) in *Regulation of Gluconeogenesis* (Söling, H.-D. & Willms, B., eds.), pp. 92–102, Academic Press Inc., New York and London
- Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflügers Arch. Ges. Physiol. Menschen Tiere* **270**, 399–412