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Effects of Dichloroacetate on the Metabolism of Glucose, Pyruvate, Acetate, 3-Hydroxybutyrate and Palmitate in Rat Diaphragm and Heart Muscle *in vitro* and on Extraction of Glucose, Lactate, Pyruvate and Free Fatty Acids by Dog Heart *in vivo*

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1. The extractions of glucose, lactate, pyruvate and free fatty acids by dog heart *in vivo* were calculated from measurements of their arterial and coronary sinus blood concentration. Elevation of plasma free fatty acid concentrations by infusion of intralipid and heparin resulted in increased extraction of free fatty acids and diminished extractions of glucose, lactate and pyruvate by the heart. It is suggested that metabolism of free fatty acids by the heart *in vivo*, as *in vitro*, may impair utilization of these substrates. These effects of elevated plasma free fatty acid concentrations on extractions by the heart *in vivo* were reversed by injection of dichloroacetate, which also improved extraction of lactate and pyruvate by the heart *in vivo* in alloxan diabetes. 2. Sodium dichloroacetate increased glucose oxidation and pyruvate oxidation in hearts from fed normal or alloxan-diabetic rats perfused with glucose and insulin. Dichloroacetate inhibited oxidation of acetate and 3-hydroxybutyrate and partially reversed inhibitory effects of these substrates on the oxidation of glucose. In rat diaphragm muscle dichloroacetate inhibited oxidation of acetate, 3-hydroxybutyrate and palmitate and increased glucose oxidation and pyruvate oxidation in diaphragms from alloxan-diabetic rats. Dichloroacetate increased the rate of glycolysis in hearts perfused with glucose, insulin and acetate and evidence is given that this results from a lowering of the citrate concentration within the cell, with a consequent activation of phosphofructokinase. 3. In hearts from normal rats perfused with glucose and insulin, dichloroacetate increased cell concentrations of acetyl-CoA, acetylcarnitine and glutamate and lowered those of aspartate and malate. In perfusions with glucose, insulin and acetate, dichloroacetate lowered the cell citrate concentration without lowering the acetyl-CoA or acetylcarnitine concentrations. Measurements of specific radioactivities of acetyl-CoA, acetylcarnitine and citrate in perfusions with [1-^{14}C]acetate indicated that dichloroacetate lowered the specific radioactivity of these substrates in the perfused heart. Evidence is given that dichloroacetate may not be metabolized by the heart to dichloroacetyl-CoA or dichloroacetylcarnitine or citrate or CO_2. 4. We suggest that dichloroacetate may activate pyruvate dehydrogenase, thus increasing the oxidation of pyruvate to acetyl-CoA and acetylcarnitine and the conversion of acetyl-CoA into glutamate, with consumption of aspartate and malate. Possible mechanisms for the changes in cell citrate concentration and for inhibitory effects of dichloroacetate on the oxidation of acetate, 3-hydroxybutyrate and palmitate are discussed.

The reactions catalysed by hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11) and pyruvate dehydrogenase (EC 1.2.4.1) are inhibited in the perfused rat heart by the oxidation of short- and long-chain fatty acids, chylomicron fatty acids, and ketone bodies (Williamson, 1964; Randle *et al.*, 1966; Ontko & Randle, 1967) and by alloxan diabetes (Park *et al.*, 1961; Randle *et al.*, 1966). The mechanisms may involve inhibition of phosphofructokinase by accumulation of citrate (Parmeggiani & Bowman, 1963; Garland *et al.*, 1963), and inhibition of pyruvate dehydrogenase through conversion into an inactive form by an unknown mechanism (Wieland *et al.*, 1971) and through end-product inhibition by an accumulation of acetyl-CoA (Garland & Randle, 1964). Further clarification of these mechanisms and of their physiological significance has been hampered by a lack of suitable metabolic inhibitors. Tubbs & Chase (1967) and Chase & Tubbs (1972) showed that 2-bromo derivatives of long-chain fatty acids may inhibit mitochondrial oxidation of fatty acids by inactivating a carnitine acyltransferase. In perfused

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hearts 2-bromo fatty acids inhibit palmitate oxidation, and reactivation of the hexokinase, phosphofructokinase and pyruvate dehydrogenase reactions has been shown in alloxan diabetes (Burges *et al.*, 1968; Randle, 1969). These inhibitors are, however, inconvenient to use because rigid control of concentration and time of exposure are necessary if other less-specific effects (e.g. interference with ATP synthesis and cessation of muscular contraction) are to be avoided.

Lorini & Ciman (1962) found that di-isopropylammonium dichloroacetate raises the respiratory quotient in alloxan-diabetic rats. This observation suggested that the compound may promote glucose oxidation and inhibit fatty acid oxidation and thus be a useful metabolic inhibitor. Preliminary experiments showed that sodium dichloroacetate stimulates oxidation of glucose *in vitro* by heart or diaphragm muscle from alloxan-diabetic rats (McAllister & Randle, 1970). Stacpoole & Felts (1970, 1971), in their studies of the effects of dichloroacetate on glucose oxidation in diabetic muscle, suggested that the compound may act by inhibiting the oxidation of long-chain fatty acids. Our own studies have suggested that other mechanisms may be involved and we describe here effects of dichloroacetate *in vitro* on the metabolism of glucose, pyruvate, acetate, 3-hydroxybutyrate and palmitate and on concentrations of metabolites in rat heart and diaphragm muscles, and on the extractions of glucose, lactate, pyruvate and free fatty acids by dog heart *in vivo*.

Materials

Enzymes, coenzymes, adenine nucleotides and other biochemicals were from Boehringer (London) Corp., London W.5, U.K. Dichloroacetic acid, L-carnitine and other chemicals (purest grade available) were from BDH Chemicals Ltd., Poole, Dorset, U.K. Insulin was a gift of Boots Pure Drug Co. Ltd., Nottingham, U.K. Heparin was from Evans Medical Ltd., Speke, Liverpool, U.K. Ion-exchange resins, Dowex 1 (AG1; formate form; X4; 200–400 mesh) and Dowex 50 (AG50W; H^+ form; X2; 200–400 mesh) were from Bio-Rad Laboratories, Richmond, Calif., U.S.A. Radioactive chemicals, [1-^{14}C]acetate, [1-^{14}C]pyruvate, 3-hydroxy[3-^{14}C]butyrate, [1-^{14}C]-palmitate, [U-^{14}C]glucose, [5-^{3}H]glucose, [1-^{14}C]-glyoxylate and trichloro[1-^{14}C]acetic acid were from The Radiochemical Centre, Amersham, Bucks., U.K. Intralipid was from Paines and Burn Ltd., Greenford, Middx., U.K. Bovine plasma albumin (fraction V) was from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K. and was freed of fatty acids as described by Garland *et al.* (1964).

Acetyl-CoA was synthesized by the method of Simon & Shemin (1953). Acetyl-L-carnitine was synthesized by the method of Fraenkel & Friedman (1957). Dichloroacetyl-L-carnitine was synthesized by a similar method; excess of dichloroacetyl chloride was removed by rotary evaporation and dichloroacetic acid was removed by passage through a column of Dowex 1 resin (formate form) and elution of dichloroacetylcarnitine with water, followed by concentration by freeze-drying. Dichloroacetylcarnitine was assayed as L-carnitine after saponification in 5M-KOH for 45min at 70°C. L-Carnitine was assayed spectrophotometrically as CoA released from acetyl-CoA by carnitine acetyltransferase by using 5,5′-dithiobis(nitrobenzoic acid) and dichloroacetate was identified by t.l.c. (see below). The product contained equimolar amounts of L-carnitine and dichloroacetyl-L-carnitine and was not further purified. Dichloro-[1-^{14}C]acetic acid was synthesized by copper reduction of trichloro[1-^{14}C]acetic acid in aqueous solution (Doughty & Derge, 1931). After removal of copper as CuS with H_2S, dichloroacetate was crystallized as the benzyl thiuronium salt and converted into dichloroacetic acid with Permutit Zeo-Karb cation-exchanger (H^+ form). The purity of benzyl thiuronium dichloroacetate was checked by melting point (173–175°C; stated 178°C) and by t.l.c. on silica-gel G plates in butan-2-one containing 0.25% (v/v) formic acid, and detected by spraying with 0.1% (w/v) Bromophenol Blue in ethanol. Dichloroacetic acid and trichloroacetic acid were located by markers and radioactivity was assayed by liquid-scintillation spectrometry after the spot had been scraped from the plate. By this method dichloro[^{14}C]acetic acid preparations contained less than 1% of trichloro-[^{14}C]acetic acid.

Methods

Heart and diaphragm experiments

Heart perfusions. Hearts were from male albino Wistar rats (250–400g) with free access to diet 41B (Oxoid Ltd., London E.C.4, U.K.). Rats were injected with heparin and Nembutal intraperitoneally before excision of the heart. The perfusion medium was bicarbonate-buffered salt solution (Krebs & Henseleit, 1932) modified by decreasing the concentration of $CaCl_2$ to one-half and by adding 0.6mM-EDTA (calcium salt). This medium was gassed with O_2+CO_2 (95:5) and contained glucose (5.5mM) and insulin (0.01i.u./ml) with other additions given in the text, figures or tables.

Hearts were perfused at 38°C by drip-through by a cannula inserted in the aorta; the perfusion pressure was 60mmHg (6.7kPa). Times of perfusion are shown in the tables or figures.

Diaphragm incubations. Hemidiaphragms were excised from male albino Wistar rats of 100–150g and incubated in 2ml of bicarbonate-buffered salt solution with the additions given in tables or figures.

Tissue extracts. For metabolite assays and measurements of specific radioactivities, hearts were rapidly frozen with a tissue clamp at the temperature of liquid N_2 and trimmed of fat. Hemidiaphragms were frozen in liquid N_2. Tissues were powdered in a percussion mortar at −70°C and a weighed amount of powder was extracted at 0°C with 5% (w/v) $HClO_4$ (approx. 2.5ml/g of powder) and centrifuged at 0°C. A measured volume of supernatant was neutralized at 0°C with saturated $KHCO_3$ and the $KClO_4$ removed by centrifugation at the same temperature. For assay of adenine nucleotides extracts were made at −10 to −15°C in 5% (w/v) $HClO_4$ in 15% (w/v) acetone and assayed within 24h.

Determinations

Glucose, lactate and pyruvate in the medium. Glucose was assayed spectrophotometrically (Slein, 1963). Lactate and pyruvate were assayed (after deproteinization with $HClO_4$) spectrophotometrically or fluorimetrically by the methods of Hohorst (1963) and Bücher *et al.* (1963).

Metabolites in heart and diaphragm. These were assayed spectrophotometrically or fluorimetrically by methods listed by Neely *et al.* (1972); additionally acetyl-L-carnitine was assayed by the method of Pearson & Tubbs (1967).

Oxygen. Concentrations of O_2 were measured in the perfusion medium at 37°C with a Clark O_2 electrode calibrated with medium saturated with O_2+CO_2 (95:5) at 37°C and with medium freed of O_2 with dithionite.

Glycolysis rate. Glycolysis rate in perfused heart was measured from the production of ^3H_2O from [5-^{3}H]glucose (Neely *et al.*, 1972).

Radioactivity. This was assayed in a Nuclear–Chicago mark I liquid-scintillation spectrometer with either toluene or methoxyethanol–toluene (2:3, v/v) containing butyl-PBD [5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] and naphthalene. Quenching corrections were determined by the channels-ratio method by an external standard.

Incorporations of radioactivity from ^{14}C-labelled compounds into CO_2 in heart and diaphragm were measured by methods given by Garland *et al.* (1964). Specific radioactivities of acetyl-CoA and citrate (acetate and oxaloacetate moieties) were measured by ion-exchange chromatography and enzymic conversions as described by Randle *et al.* (1970). For measurement of the specific radioactivitiy of acetyl-L-carnitine this substance was assayed in heart extracts. Carrier acetyl-L-carnitine was then added and the concentration assayed again to give the dilution factor. Acetyl-L-carnitine was then purified for assay of radioactivity by applying a measured volume of extract to a column (20cm × 1cm) of Dowex 1 (formate form) and by specific elution with 100ml of water.

Experiments in dogs in vivo

Animals. Beagles weighing 9–11kg were fed daily at 16:00h; any residual food was removed at 17:00h. The diet consisted of Full o' Pep dog food (Armitage Brothers, Colwick, Notts., U.K.). At least 2 weeks before any experiments were performed a carotid loop was fashioned in each animal for collection of arterial blood samples. In each experiment the exteriorized carotid artery enclosed in a skin flap was cannulated with a hypodermic needle no. 1 kept patent by flushing with 0.9% NaCl containing heparin. On the day of study the animals were anaesthetized by intravenous administration of sodium Amytal commencing at 11:00h. An endotracheal tube was passed and a flow of pure O_2 maintained. A cardiac catheter (no. 6 or 7) was passed under screening control into the coronary sinus and the final position of the catheter checked by injection of contrast medium. Recovery was allowed at the end of the experiment except for alloxan-diabetic dogs.

Procedures. Three types of experimental design were employed.

Group 1. Control experiments were carried out in five dogs to ensure that steady-state conditions had been achieved. In these experiments 0.9% NaCl was infused throughout a 3h period at a rate of 40ml/h. No samples were taken during the first hour of infusion. Simultaneous arterial and coronary sinus blood samples were then taken at 1, 1.25, 1.5 and at 2.5, 2.75 and 3h.

Group 2. These experiments were designed to investigate effects of intralipid plus heparin with and without dichloroacetate. In seven dogs 0.9% NaCl was infused over the first 1.5h and blood samples were taken at 1, 1.25 and 1.5h. Heparin was then injected intravenously (1000 units) and a constant infusion of intralipid (40ml/h) and heparin (400 units/h) given from 1.5 to 3h; blood samples were taken at 2.5, 2.75 and 3h. In three of the dogs a single dose of sodium dichloroacetate (3g) was administered at the end of 3h and intralipid and heparin infusions were continued for a further 60min; blood samples were taken at 5, 10, 20, 30, 45 and 60min after the injection of dichloroacetate.

Group 3. In these experiments two dogs were rendered diabetic by intravenous injection of alloxan (70mg/kg) and used after 48h. Infusion of 0.9% NaCl was made and blood samples were taken at 1, 1.25 and 1.5h. Sodium dichloroacetate (3g) was then given intravenously and further blood samples were taken at 5, 10, 20, 30, 45 and 60min.

Blood samples. Blood samples were collected in heparinized syringes and placed in centrifuge tubes at 0°C. For assay of glucose, lactate and pyruvate whole blood was deproteinized within 5min of collection with 0.6M-perchloric acid; the extracts obtained by centrifuging were neutralized with saturated $KHCO_3$. Samples of plasma for estimation

of free fatty acids were rapidly separated by using a refrigerated centrifuge and the fatty acids extracted by using a modification of the method of Dole (Trout *et al.*, 1960). Plasma free fatty acids were estimated in duplicate by the spectrophotometric method of Duncombe (1962). For this purpose the heptane extracts of fatty acids were dried in a stream of N_2 and the fatty acids redissolved in chloroform. Glucose, lactate and pyruvate were estimated spectrophotometrically by methods given above. Oxygen saturation was measured with an O_2 saturation meter OSM1 (Radiometer, Copenhagen, Denmark).

Calculations. Blood concentrations, arteriovenous differences and percentage extractions on passage through the heart are given in mmol/l or are calculated from concentrations expressed in this way. Results for each group are given in the tables as the means ± S.E.M. and levels of statistical significance are calculated from Student's *t* test. The percentage extraction of a substrate was calculated from (arterial blood concentration − coronary sinus blood concentration)/arterial blood concentration.

Results

Effects of elevated plasma free fatty acids and of dichloroacetate on extractions of glucose, lactate, pyruvate and free fatty acids by dog heart in vivo

Table 1 shows the results of control experiments in five dogs to establish the existence of steady-state concentrations and arteriovenous differences across the heart during 3 h of infusion with 0.9% NaCl. The experimental design involved measurements on blood samples taken at 1, 1.25 and 1.5 h (period 1) and at 2.5, 2.75 and 3 h (period 2). There were no significant differences between the mean values in each of the two periods for arterial blood concentrations of glucose, lactate or pyruvate or plasma concentrations of free fatty acids or for arteriovenous concentration differences for these substances. Table 2 shows the results of experiments in seven dogs in which the experimental design was varied by a single injection of heparin at the beginning of period 2 and infusion of intralipid and heparin in place of 0.9% NaCl in period 2. Elevation of plasma free fatty acids by intralipid and heparin in the second period had no effect on arterial blood concentrations of glucose, lactate or pyruvate. As shown by the arteriovenous differences, the myocardial extractions of glucose, lactate and pyruvate were inhibited 60–85% at the higher concentration of free fatty acids. Table 3 shows the results of experiments similar to those shown in Table 2, but with the addition of a third period in which measurements were continued for a further hour after the injection of a single dose of sodium dichloroacetate (4 g). As in Table 2 infusion of intralipid and heparin diminished extraction of glucose, lactate and pyruvate by the heart. After injection of dichloroacetate blood concentrations of lactate and pyruvate fell significantly and the myocardial extractions of glucose, lactate and pyruvate increased, though the change achieved statistical significance only for glucose. However, the arterial concentrations

Table 1. *Arterial blood concentrations of glucose, lactate, pyruvate and free fatty acids, arteriovenous differences and percentage extractions by dog heart in vivo during intravenous infusions with 0.9% NaCl*

Blood and plasma concentrations were measured in samples withdrawn from the external carotid artery (arterial) or the coronary sinus in normal dogs anaesthetized with sodium Amytal (see the Methods section) and infused throughout with 0.9% NaCl (40 ml/h). Blood samples were taken at 1, 1.25 and 1.5 h after the beginning of infusion (period 1) and at 2.5, 2.75 and 3 h (period 2). The values shown are the mean values for the three samples taken in each period in five dogs. Significance of difference between means in period 1 and period 2: $P > 0.05$ in all instances.

	Mean ± S.E.M.	
	Period 1	Period 2
Infusion	0.9% NaCl	0.9% NaCl
Arterial blood glucose (mM)	5.42 ± 0.4	5.19 ± 0.38
Arteriovenous difference	0.36 ± 0.28	0.32 ± 0.11
% extraction	6.83 ± 1.27	6.50 ± 1.25
Arterial blood lactate (mM)	1.63 ± 0.4	1.75 ± 0.28
Arteriovenous difference	0.82 ± 0.53	1.0 ± 0.17
% extraction	53.0 ± 2.8	55.0 ± 3.3
Arterial blood pyruvate (mM)	0.122 ± 0.029	0.114 ± 0.04
Arteriovenous difference	0.085 ± 0.02	0.084 ± 0.02
% extraction	65.0 ± 3.5	69.0 ± 4.9
Arterial plasma free fatty acids (mM)	0.495 ± 0.060	0.505 ± 0.069
Arteriovenous difference (mM)	0.130 ± 0.024	0.133 ± 0.025

Table 2. *Effect of infusion of intralipid and heparin on arterial concentrations, arteriovenous differences and percentage extractions of glucose, lactate, pyruvate and free fatty acids by dog heart in vivo*

The experimental conditions and times of sampling were as in Table 1 except that intralipid (40ml/h) and heparin (1000i.u., by single injection, and 400i.u./h) were given over period 2 in place of 0.9% NaCl. The values shown are the mean values for the three samples taken in each period in seven dogs. Significance of differences between mean values in period 1 and period 2 (from paired observations): $^{**}P<0.01$. For other differences, $P>0.05$.

	Mean ± S.E.M.	
	Period 1	Period 2
Infusion	0.9% NaCl	Intralipid and heparin
Arterial blood glucose (mM)	5.22 ±0.29	5.13 ±0.31
Arteriovenous difference	0.31 ±0.09	0.13 ±0.05**
% extraction	6.0 ±1.0	2.5 ±0.70**
Arterial blood lactate (mM)	1.05 ±0.20	0.83 ±0.83
Arteriovenous difference	0.44 ±0.12	0.12 ±0.03**
% extraction	42.2 ±3.6	12.8 ±2.0**
Arterial blood pyruvate (mM)	0.079±0.017	0.053±0.011
Arteriovenous difference	0.036±0.011	0.006±0.004**
% extraction	41.0 ±5.3	4.7 ±5.2**
Arterial plasma free fatty acids (mM)	0.56 ±0.048	1.47 ±0.15**
Arteriovenous difference	0.084±0.009	0.25 ±0.035**

Table 3. *Effect of sodium dichloroacetate on arterial concentrations, arteriovenous differences and percentage extractions of glucose, lactate, pyruvate and free fatty acids by dog heart in vivo during infusion of heparin and intralipid*

The experimental conditions and times of sampling during periods 1 and 2 were as in Table 2. In these experiments infusion of intralipid was continued for a third period of 1 h at the same rate as in period 2. A single injection of sodium dichloroacetate (4g) was given intravenously at the beginning of period 3 and further samples were taken after 5, 10, 20, 30, 45 and 60 min. The values shown are the mean values for three samples in periods 1 and 2 and for six samples in period 3 in three dogs. Significance of differences between mean values in periods 1 and 2, and 2 and 3 (from paired observations): $^{*}P<0.05$, $^{**}P<0.01$. For other differences, $P>0.05$.

	Period 1	Period 2	Period 3
Infusion	0.9% NaCl	Intralipid + heparin	Intralipid (after injection of dichloroacetate) + heparin
Arterial blood glucose (mM)	5.64	5.17**	5.10
Arteriovenous difference	0.49	0.17	0.37**
% extraction	9.2	4.2*	7.9**
Arterial blood lactate (mM)	0.65	0.66	0.30**
Arteriovenous difference	0.35	0.14*	0.17
% extraction	53	22**	57**
Arterial blood pyruvate (mM)	0.043	0.036	0.010**
Arteriovenous difference	0.024	−0.003*	0.010
% extraction	33	−10*	98**
Arterial plasma free fatty acids (mM)	660	1866	1622
Arteriovenous difference	100	345	266

of lactate and pyruvate fell after injection of dichloroacetate and Goodale & Hackel (1953) have shown that the utilization of these substrates by dog heart is proportional to their arterial concentrations. It seemed appropriate therefore to take into account the effect of changing arterial concentrations by comparing the percentage extractions of lactate and pyruvate by the heart before and after dichloroacetate injection. These were increased significantly by dichloroacetate. The effect on pyruvate extraction was

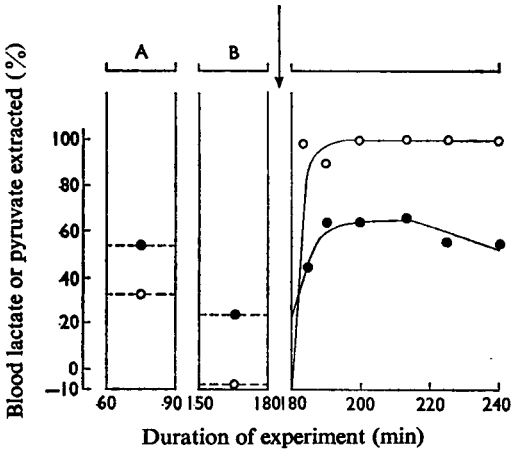


Fig. 1. *Effect of injection of dichloroacetate on myocardial extractions of lactate* (●) *and pyruvate* (○) *in dogs infused with intralipid+heparin*

The values shown in the periods of infusion of 0.9% (A) NaCl and of intralipid+heparin (B: before dichloroacetate injection) are the means of individual values at 60, 75 and 90min and at 150, 165 and 180min. Dichloroacetate (3g) was given as a single intravenous injection at the time marked by the arrow. For other details see the Methods section. The values shown are the mean values for seven dogs.

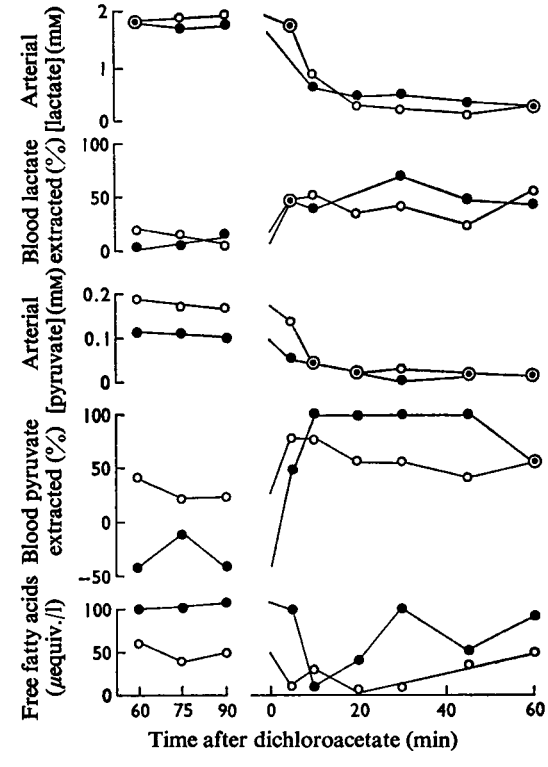


Fig. 2. *Effect of dichloroacetate on blood concentrations and myocardial extractions in alloxan-diabetic dogs in vivo*

In the basal period 0.9% NaCl was infused and blood samples were collected from two alloxan-diabetic dogs at 60, 75 and 90min. Sodium dichloroacetate (3g) was then given intravenously and further collections and analyses were made as shown. ●, Dog 1; ○, dog 2.

so marked that none could be detected in plasma of blood taken from the coronary sinus after injection of dichloroacetate (lower limit of assay approx. 10% of pyruvate concentration before dichloroacetate injection). The concentration of plasma free fatty acids and the extraction of free fatty acids by the heart did not change significantly with dichloroacetate. As shown in Fig. 1 the action of dichloroacetate was rapid, increased extractions of lactate and pyruvate being detected within 5min.

Fig. 2 shows the effect of a single injection of dichloroacetate (4g) in two alloxan-diabetic dogs (blood glucose concentrations 360mg/100ml in the unfed state). The arterial concentration of glucose and the arteriovenous concentration differences for glucose showed no consistent change with dichloroacetate (not shown). The arterial concentrations of lactate and of pyruvate fell substantially and there were marked and rapid increases in the percentage extractions of these substrates by the heart. Dichloroacetate did not change the plasma concentration of free fatty acids but the arteriovenous difference fell from 75μmol/l before dichloroacetate to 20μmol/l within 10min of its injection.

The percentage saturation of arterial blood with O_2 (98–99%) did not vary during any of these experiments. The heart rate (106 beats/min) was slowed (to 72–90) after dichloroacetate but was otherwise unchanged during these experiments.

Effects of dichloroacetate on the oxidations of glucose, palmitate, acetate, DL-*3-hydroxybutyrate and pyruvate by rat hemidiaphragms and rat heart in vitro*

The oxidation of [U-^{14}C]glucose to $^{14}CO_2$ by hemidiaphragms from normal rats was unaffected by dichloroacetate (10mM) but the inhibition of glucose oxidation by alloxan diabetes or by DL-3-hydroxybutyrate was completely reversed (Table 4). The oxidation of [1-^{14}C]acetate, DL-3-hydroxy[3-^{14}C]-butyrate and of [1-^{14}C]palmitate to $^{14}CO_2$ by hemidiaphragms from normal rats was inhibited by dichloroacetate (Table 5). The oxidation of [1-^{14}C]-

Table 4. *Effect of dichloroacetate* (10 mM) *on the conversion of* [U-^{14}C]*glucose into* $^{14}CO_2$ *in rat diaphragm muscle*

Hemidiaphragms from normal fed or alloxan-diabetic rats were incubated for 1 h in medium containing [U-^{14}C]-glucose (5.5 mM; 5 nCi/μmol) and insulin ($20–50 \times 10^{-3}$ i.u./ml) and other additions as shown. Metabolism was arrested by addition of 8 μequiv. of H_2SO_4 and the CO_2 liberated was collected in Hyamine for assay of radioactivity. Six hemidiaphragms were incubated in each group. Means ± S.E.M. are presented. Significance of difference from control: ***$P<0.001$.

Rats	Other additions to medium	Production of $^{14}CO_2$ (d.p.m./g of wet muscle per h) Control	Dichloroacetate
Normal	None	19785 ± 1533	18722 ± 1447
Normal	3-Hydroxybutyrate (5 mM)	9252 ± 1024	18169 ± 1030***
Diabetic	None	5744 ± 953	20126 ± 1466***

Table 5. *Effects of dichloroacetate* (10 mM) *on the rate of conversion of* [1-^{14}C]*acetate,* 3-*hydroxy*[3-^{14}C]*butyrate,* [1-^{14}C]*palmitate and* [1-^{14}C]*pyruvate into* $^{14}CO_2$ *in rat diaphragm*

For conditions of incubation see Table 4. Media contained glucose (5.5 mM) and insulin (50×10^{-3} i.u./ml) except in the experiment with palmitate. Other additions are as given. Means ± S.E.M. are presented. Significance of difference from control: ***$P<0.001$. Six hemidiaphragms were incubated in each group.

Rats	Other additions to medium	Production of $^{14}CO_2$ (d.p.m./g wet muscle per h) Control	Dichloroacetate
Normal	Acetate (5 mM; 5 nCi/μmol)	92474 ± 9330	55591 ± 4860***
Normal	3-Hydroxybutyrate (5 mM; 5 nCi/μmol)	37355 ± 1505	22581 ± 1248***
Normal	Palmitate (0.6 mM on 2% albumin; 5 nCi/μmol)	1158 ± 50	541 ± 63***
Diabetic	Pyruvate (5 mM; 5 nCi/μmol)	45978 ± 7859	95050 ± 9430***

pyruvate by diaphragm muscle from alloxan-diabetic rats was increased by dichloroacetate (Table 5).

In the perfused rat heart dichloroacetate (10 mM) increased the rate of oxidation of [U-^{14}C]glucose in hearts from normal or alloxan-diabetic rats and increased the rate of oxidation of [1-^{14}C]pyruvate in hearts from normal rats. Dichloroacetate (10 mM) inhibited the oxidation of [1-^{14}C]acetate and DL-3-hydroxy[3-^{14}C]butyrate and reversed the inhibitory effects of acetate and DL-3-hydroxybutyrate on the oxidation of [U-^{14}C]glucose in hearts from normal rats (Table 6). Table 7 shows that inhibition of acetate oxidation can occur with 0.1 mM-dichloroacetate and that 1 mM- and 10 mM-dichloroacetate produce approximately the same degree of inhibition.

The inhibitory effect of dichloroacetate on acetate oxidation was reversible. Hearts were perfused for 16 min with medium containing glucose (5.5 mM), insulin (20×10^{-3} i.u./ml) and dichloroacetate (1 mM). They were then perfused for 5 min with medium containing in addition [1-^{14}C]acetate (5 mM; 5 nCi/μmol) ± dichloroacetate and production of radioactive CO_2 was measured. When dichloroacetate was present during the 5 min of perfusion the rate of production of $^{14}CO_2$ was 22795 ± 1500 d.p.m./5 min per g of dry muscle. When dichloroacetate was absent the rate of $^{14}CO_2$ production was 111123 ± 2100 (mean ± S.E.M. for four hearts in each group; $P<0.001$ for difference).

In hearts from normal rats perfused with media containing glucose, insulin and acetate, dichloroacetate approximately doubled the rate of glycolysis and decreased the output of lactate by about 50% (Table 8). In further experiments (not shown) it was found that dichloroacetate did not change the concentration of glycogen in hearts perfused with glucose, insulin and acetate. The rate of glucose oxidation calculated from the difference (glycolytic rate − lactate output) was increased substantially by dichloroacetate in perfusions with glucose, insulin and acetate (Table 8). Measurements of consumption of O_2 showed a small increase with dichloroacetate (from 22.5 ± 1 to 25.2 ± 0.7 μmol/min per g dry wt.), which was not statistically significant.

Further evidence for an inhibitory effect of dichloroacetate on the oxidation of acetate through

Table 6. *Effect of dichloroacetate (10 mM) on conversion of ^{14}C-labelled substrates into $^{14}CO_2$ in perfused rat heart*

Hearts from normal fed or alloxan-diabetic rats were pre-perfused for 10 min with medium containing glucose (5.5 mM) and insulin (20×10^{-3} i.u./ml) with or without dichloroacetate (10 mM) and then were switched to medium containing in addition ^{14}C-labelled substrates as shown and perfused for 5 min. During the perfusion period medium issuing from the heart was collected at 0°C under heptane and subsequently assayed for $^{14}CO_2$. Significance of differences from control: $^{**}P<0.01$; $^{*}P<0.05$. Means ± S.E.M. are presented with the number of hearts perfused in each group shown in parentheses.

Rats	Other additions to medium	$10^{-3} \times$ Production of $^{14}CO_2$ (d.p.m./5 min per g of dry muscle) Control	Dichloroacetate
Diabetic	[U-^{14}C]Glucose (5 nCi/μmol)	11.4 ± 8.3	60.0 ± 9.2** (4)
		19.2 ± 14.0	89.4 ± 17.0** (4)
Normal	[U-^{14}C]Glucose (5 nCi/μmol)	27.8 ± 2.3	37.0 ± 2.7* (10)
	[U-^{14}C]Glucose (5 nCi/μmol) with acetate (5 mM)	7.7 ± 0.5	37.5 ± 1.9** (6)
	[U-^{14}C]Glucose (5 nCi/μmol) with 3-hydroxybutyrate (5 mM)	10.0 ± 0.3	17.3 ± 1.7** (4)
	[1-^{14}C]Acetate (5 mM; 5 nCi/μmol)	137.6 ± 1.2	44.0 ± 1.4** (14)
	3-Hydroxy[3-^{14}C]butyrate (5 mM; 5 nCi/μmol)	39.3 ± 0.4	20.0 ± 1.6** (5)
	[1-^{14}C]Pyruvate (5 mM; 5 nCi/μmol)	93.6 ± 7.8	127.9 ± 5.2** (5)

Table 7. *Effect of various concentrations of dichloroacetate on the rate of conversion of [1-^{14}C]acetate into $^{14}CO_2$*

Hearts from normal fed rats were pre-perfused for 10 min with media containing glucose (5.5 mM) and insulin (20×10^{-3} i.u./ml) with or without dichloroacetate at the concentrations shown. Perfusion was then continued for a further 5 min with medium containing in addition acetate (5 mM; 5 nCi/μmol) and perfusate was collected for assay of $^{14}CO_2$. Means ± S.E.M. are presented. Significance of difference from zero control: $^{***}P<0.001$. Four hearts were perfused in each group.

Concn. of dichloroacetate (mM)	Production of $^{14}CO_2$ (d.p.m./5 min per g dry wt.)
0	154830 ± 1630
0.1	99210 ± 1340***
1	51496 ± 1590***
10	64398 ± 1512***

the tricarboxylate cycle has been obtained from measurements of the specific radioactivities of acetyl-CoA, acetylcarnitine and citrate (acetate and oxaloacetate moieties) in hearts from normal rats perfused with [1-^{14}C]acetate. Dichloroacetate approximately halved the specific radioactivities of these tricarboxylate cycle metabolites (Table 10).

By visual inspection dichloroacetate had no effect on either the rate or the force of cardiac contraction in the perfused rat heart.

Effects of dichloroacetate on concentrations of metabolites in perfused rat heart

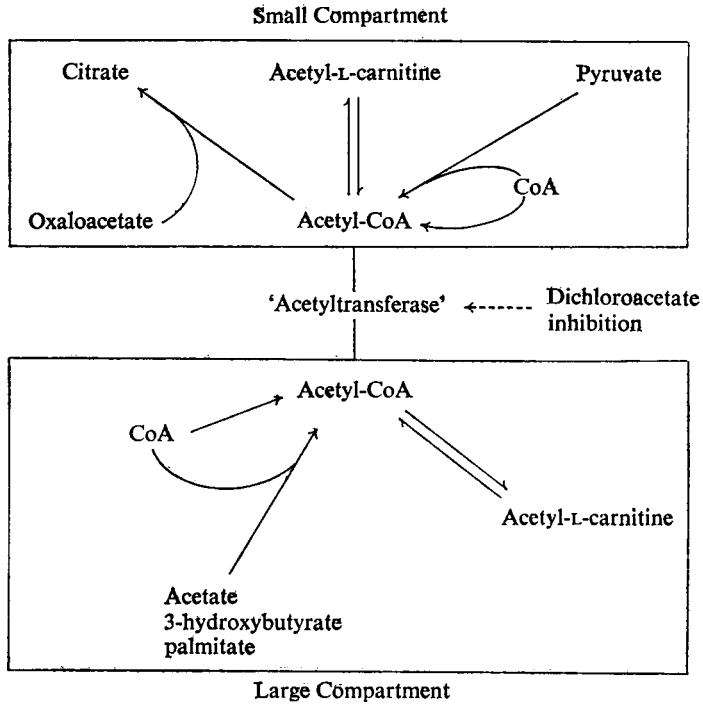
In glucose perfusions, dichloroacetate (at 10 mM, as shown, or at 1 mM, not shown) markedly increased the concentrations of acetyl-CoA and acetylcarnitine in hearts from normal rats. Concentration of glutamate was also increased and those of pyruvate, aspartate and malate decreased, suggesting increased oxidation of pyruvate into acetyl-CoA and increased incorporation of acetyl-CoA into glutamate, with the oxaloacetate required being derived from aspartate (by transamination with 2-oxoglutarate) and malate. Concentrations of citrate, glucose 6-phosphate and adenine nucleotides were unchanged (Table 9). Acetate perfusion led to the expected changes in metabolite concentrations: increases in acetyl-CoA and acetylcarnitine; increases in citrate and glutamate with provision of oxaloacetate through a decrease in aspartate; an increase in glucose 6-phosphate as a result of inhibition of phosphofructokinase and glycolysis by citrate (Table 9; Garland & Randle, 1964; Williamson, 1965; Bowman, 1966; Randle *et al.*, 1970). In acetate perfusions, dichloroacetate lowered heart citrate concentration and this was associated with accelerated glycolysis and lowered glucose 6-phosphate concentration, indicating activation of phosphofructokinase (Tables 8 and 9). The concentrations of acetyl-CoA, acetylcarnitine, glutamate, aspartate and adenine nucleotides were not significantly altered by dichloroacetate in acetate perfusions (Table 9). Dichloroacetate also lowered concentrations of citrate and glucose 6-phosphate during perfusion with DL-3-hydroxybutyrate.

In diaphragms from normal rats incubated with

Table 8. *Effect of dichloroacetate* (10mM) *on the rate of glycolysis, lactate output and glucose oxidation in perfused rat heart*

Hearts from normal fed rats were perfused for 15min with media containing glucose (5.5mM), insulin (20×10^{-3}i.u./ml) and acetate (5mM) with or without dichloroacetate. Medium was collected for estimations of lactate output and ^3H_2O production from [5-^{3}H]glucose from 8 to 12 and 12 to 15min. For details see the Methods section. Results for perfusions with glucose + insulin only are taken from Neely *et al.* (1972). Five hearts were perfused in each group. Significance of differences from control: $*P<0.05$; $**P<0.01$.

Perfusion medium ...	Glucose+acetate		Glucose+acetate+dichloroacetate		
Collection time (min) ...	8–12	12–15	8–12	12–15	Glucose
Rate (μmol of glucose equivalent/min per g dry wt.)					
Glycolysis	2.0 ±0.22	2.0 ±0.18	3.28±0.29**	4.42±0.34**	6.5±0.3
Lactate output	1.80±0.24	2.18±0.42	1.14±0.08*	1.45±0.15	2.3±0.24
Glucose oxidation (glycolysis−lactate output)	0.20±0.21	−0.18±0.28	2.14±0.13**	2.97±0.21**	3.7



Scheme 1. *Two-compartment model of acetyl-CoA metabolism*

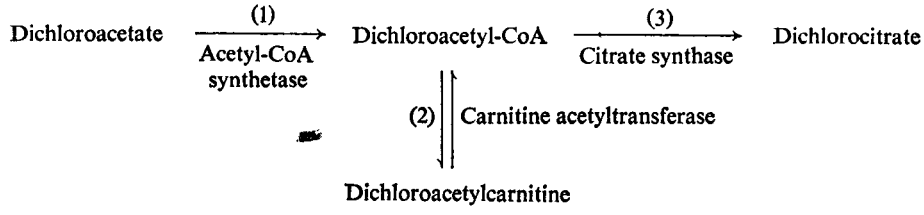
The postulated 'acetyltransferase' forms acetyl-CoA in either compartment from CoA in that compartment and acetyl groups transferred from the other compartment.

glucose and insulin, dichloroacetate increased the concentration of acetyl-CoA (from 0.013±0.006 to 0.151±0.004μmol/g dry wt.) and that of glutamate (from 5.3±0.3 to 8.3±0.7μmol/g dry wt.) and decreased the concentrations of citrate (from 0.96±0.06 to 0.46±0.07μmol/g dry wt.) and aspartate (from

Table 9. *Effect of dichloroacetate* (10 mM) *on tissue concentrations of metabolites in perfused rat heart*

Hearts were perfused for 15 min with medium containing glucose (5.5 mM) and insulin (20×10^{-3} i.u./ml) with or without dichloroacetate. In perfusions with acetate (5 mM) or 3-hydroxybutyrate (5 mM) these substrates were perfused together with glucose and insulin with or without dichloroacetate after 10 min of pre-perfusion with media containing glucose and insulin with or without dichloroacetate. At the end of perfusion hearts were frozen, extracted and assayed (see the Methods section). Means ± S.E.M. are presented for perfusion with glucose, insulin and the indicated additions. There were six hearts in each group. $*P<0.05$, $**P<0.01$.

	Concn. of metabolite (μmol/g of dry heart)					
Metabolite	Control	Dichloroacetate	Acetate	Acetate + dichloroacetate	3-Hydroxybutyrate	3-Hydroxybutyrate + dichloroacetate
Acetyl-CoA	0.01 ± 0.003	0.09 ± 0.008**	0.15 ± 0.009	0.18 ± 0.011	0.16 ± 0.01	0.19 ± 0.02
Acetylcarnitine	<0.06	1.6 ± 0.1**	2.77 ± 0.25	3.45 ± 0.62	—	—
Citrate	1.0 ± 0.03	1.2 ± 0.06	2.62 ± 0.10	0.78 ± 0.07	2.74 ± 0.07	0.74 ± 0.06**
Malate	0.51 ± 0.03	0.29 ± 0.01	0.79 ± 0.04	0.32 ± 0.02**	—	—
Aspartate	12.0 ± 0.57	4.7 ± 0.29**	—	—	—	—
Glutamate	18.2 ± 0.37	25.8 ± 1.74**	—	—	—	—
Glucose 6-phosphate	1.1 ± 0.05	1.1 ± 0.05	1.8 ± 0.14	1.2 ± 0.13**	1.5 ± 0.07	1.2 ± 0.02*
ATP	21.7 ± 1.4	22.0 ± 0.09				
ADP	5.5 ± 0.72	4.8 ± 0.45				
Pyruvate	0.29 ± 0.017	0.10 ± 0.014**				



Scheme 2. *Possible pathway of metabolism of dichloroacetate*

Table 10. *Effects of dichloroacetate* (10mM or 1mM) *on the tissue concentrations and specific radioactivities of acetyl-CoA, acetylcarnitine and citrate in hearts perfused with glucose, insulin and* [1-^{14}C]*acetate*

Hearts were pre-perfused for 5min with medium containing glucose (5.5mM) and insulin (20×10^{-3}i.u./ml) with or without dichloroacetate (1 or 10mM) and were then perfused for a further 5min with medium also containing sodium acetate (5mM; 60nCi/μmol). Hearts were then frozen with the tissue clamp and extracted and analysed as described in the Methods section. Means±S.E.M. are presented with the number of observations shown in parentheses. *$P<0.05$, **$P<0.01$.

	Concn. of metabolite in heart (μmol/g dry wt.)		
Perfusion	Acetyl-CoA	Acetylcarnitine	Citrate
Control	0.12±0.02 (4)	2.60±0.43 (2)	2.38±0.43 (4)
Dichloroacetate	0.18±0.02* (4)	3.1 ±0.88 (2)	0.82±0.17** (4)

	Sp. radioactivity (% of acetate in medium)			
	Acetyl-CoA	Acetylcarnitine	Citrate (acetate moiety)	Citrate (malate moiety)
Control	95±7 (4)	91±3 (3)	75±10 (5)	50±6 (5)
Dichloroacetate	50±8** (4)	61±4** (3)	45±2* (5)	29±3** (5)

2.2±0.05 to 1.3±0.08μmol/g dry wt.). Dichloroacetate also lowered the citrate concentration in diaphragms from alloxan-diabetic rats (from 1.02± 0.15 to 0.43±0.05μmol/g dry wt.).

The fall in citrate concentration caused by dichloroacetate in rat heart during acetate perfusions may provide an explanation for its effects on the glycolytic rate. However, the results of these measurements of metabolite concentrations suggest no obvious mechanism for the fall in citrate concentration caused by dichloroacetate in acetate perfusions nor for the failure of citrate concentration to rise in parallel with acetyl-CoA and glutamate concentrations in glucose perfusions. A number of possible explanations for the fall in citrate concentration have been considered. Evidence bearing on two of these possibilities is considered in the following sections and other possibilities are considered in the general discussion.

Effects of dichloroacetate on the specific radioactivities of acetyl-CoA, acetylcarnitine and citrate in rat heart

Scheme 1 shows a model capable of accounting for the effects of dichloroacetate on the concentrations of acetyl-CoA and citrate. The model postulates two compartments of CoA and carnitine in rat heart mitochondria. The small compartment contains pyruvate dehydrogenase, citrate synthase and carnitine acetyltransferase and can form acetyl-CoA, acetylcarnitine and citrate. The large compartment can form acetyl-CoA and acetylcarnitine from acetate, palmitate and ketone bodies, but not from pyruvate; it lacks citrate synthase. It is postulated that the two pools of acetyl-CoA and acetylcarnitine are equilibrated through an 'acetyltransferase'. Citrate synthesis from acetate would involve synthesis of acetyl-CoA in the large compartment, acetyl transfer to form acetyl-CoA in the small compartment, followed by citrate synthesis in the small compartment. The model postulates inhibition of the 'acetyltransferase' by dichloroacetate. In perfusions with glucose, acetate and dichloroacetate, acetyl-CoA is synthesized in the large compartment but acetyl transfer into the small compartment for synthesis of citrate is inhibited; citrate is thus synthesized from acetyl-CoA formed by the oxidation of pyruvate in the small compartment. The concentration of acetyl-CoA in the large compartment would thus be high because

of synthesis from acetate. The concentration in the small compartment would remain low and hence citrate concentration would be low. In perfusions with glucose, dichloroacetate inhibits transfer of acetyl groups between the two compartments. Acetyl-CoA in the small compartment is formed from pyruvate and metabolized through citrate. Acetyl-CoA is synthesized at a slow rate in the large compartment from palmitate formed by the breakdown of endogenous triglyceride and accumulates, thus leading to an elevated concentration of tissue acetyl-CoA. The idea of two pools of acetyl-CoA has also been postulated for rat heart mitochondria by Pearson & Tubbs (1967) to account for metabolic effects of propionate, and by Fritz (1967).

If the model is correct then in perfusions with [1-^{14}C]acetate, dichloroacetate should lower the specific radioactivity of citrate but not that of acetyl-CoA or of acetylcarnitine. Thus by inhibiting the postulated 'acetyltransferase' dichloroacetate would impair the incorporation of acetate carbon atoms into citrate but not the accumulation of [1-^{14}C]acetyl-CoA in the large compartment. Citrate would be synthesized principally from unlabelled acetyl-CoA formed from pyruvate in the small compartment. As shown in Table 10, dichloroacetate lowered the specific radioactivities of acetyl-CoA, acetylcarnitine and citrate by comparable amounts in perfusions with [1-^{14}C]acetate. The model was therefore not supported experimentally.

Metabolism of dichloroacetate by perfused rat heart

We have considered the possibility that dichloroacetate may be metabolized by rat heart according to Scheme 2. If this scheme operates then dichloroacetyl-CoA and dichloroacetylcarnitine could be included in enzymic assays for acetyl-CoA and acetylcarnitine. The formation of dichlorocitrate could lead to the disappearance of aspartate and to the accumulation of glutamate (through aspartate aminotransferase). If dichlorocitrate was not reactive in the enzymic assay for citrate then the apparent lack of parallelism between acetyl-CoA, citrate and glutamate concentrations could be explained. We have attempted to explore this pathway as follows.

Reactions (2) and (3) in Scheme 2 were tested by incubation of dichloroacetyl-L-carnitine (0.5μmol) with CoA, malate, NAD^+, carnitine acetyltransferase and citrate synthase under conditions described for the assay of acetyl-L-carnitine by Pearson & Tubbs (1967). If reactions (2) and (3) were operative then the conversion of dichloroacetyl-L-carnitine into dichlorocitrate should lead to oxidation of malate and reduction of NAD^+ to NADH with an increase in E_{340}. In experiments with 0.3μmol of dichloroacetyl-L-carnitine no change in E_{340} was detected (expected change 0.62); the subsequent addition of 0.1μmol of acetyl-L-carnitine led to the expected change in E_{340}. This observation appears to preclude reactions (2) and (3) and also shows that dichloroacetyl-L-carnitine is not an inhibitor of carnitine acetyltransferase. This observation does not preclude reaction (2) alone or reaction (3) alone. Attempts to prepare dichloroacetyl-CoA by chemical methods for acetyl-CoA (Simon & Shemin, 1953) were unsuccessful. Attempts to prepare dichloroacetyl-CoA or to demonstrate formation of dichloroacetyl-CoA by reaction (1) of the scheme with ox heart acetyl-CoA synthetase were also unsuccessful.

The metabolism of dichloro[1-^{14}C]acetate was investigated in perfused rat heart. Perfusions were made with media containing glucose (5.5mM), insulin (20×10^{-3}i.u./ml) and dichloro[1-^{14}C]acetate (either 10mM, 6nCi/μmol or 1mM, 46nCi/μmol). The heart produced very little radioactive CO_2 from dichloro-[1-^{14}C]acetate (the rate was only 0.3% of that seen with [1-^{14}C]acetate). After perfusion hearts were frozen and perchloric acid extracts prepared and analysed for radioactive components by chromatography on a column (20cm$\times$1cm) of Dowex 1 (formate form) employing sequential elution with water (100ml), an exponential gradient of 250ml of 1M-formic acid into 250ml of water, an exponential gradient of 250ml of 1M-ammonium formate into 250ml of 0.63M-formic acid, a step of 100ml of 4M-ammonium formate, and a step of 300ml of 1M-HCl. As a control a similar column was run with a sample of dichloro[1-^{14}C]acetate used in the perfusion. Dichloroacetate was eluted by the 1M-HCl step at the end and approx. 75% of the applied radioactivity (0.1μCi) was eluted by this step from applied dichloroacetate or perchloric acid extract of heart. The water wash (which would be expected to contain dichloroacetylcarnitine) yielded 0.15% of the radioactivity from dichloroacetate columns and 0.14–0.5% from columns with heart extracts. The remaining fractions (formic acid gradient, ammonium formate gradient, ammonium formate step) yielded 2% of the radioactivity applied in dichloroacetate and 1.2–3% of the radioactivity applied in heart extracts.

The water wash from the Dowex 1 (formate form) column to which was applied heart extract perfused with 1mM-dichloroacetate (46nCi/μmol) contained 0.6μmol of acetylcarnitine by enzyme assay and 0.4nCi of ^{14}C. Thus if this radioactivity represented dichloro[1-^{14}C]acetylcarnitine it would correspond to only 0.01μmol of dichloroacetate. It seems unlikely therefore that dichloroacetate is metabolized to dichloroacetylcarnitine in rat heart; taken in conjunction with the enzyme experiments mentioned above it seems unlikely that dichloroacetate is metabolized by the pathway shown in Scheme 2.

Discussion

Effects of intralipid and heparin infusion and of dichloroacetate injection on myocardial extractions in dogs in vivo

Inhibitory effects of free fatty acids on the uptake and oxidation of glucose by rat heart *in vitro* have been described by a number of investigators and were first reported by Shipp *et al.* (1961). Evidence for similar effects caused by plasma free fatty acids *in vivo* have been lacking. Lassers *et al.* (1971) reported a negative correlation between myocardial extractions of glucose, lactate and pyruvate and the arterial plasma free fatty acid concentration in man, which they interpret as showing that plasma free fatty acids can suppress glucose uptake and pyruvate oxidation by the human heart *in vivo*. Chylomicron fatty acids can also inhibit uptake and oxidation of glucose by rat heart *in vitro* (Ontko & Randle, 1967). In the present study effects of elevating plasma free fatty acids (and presumably plasma triglyceride) by infusion of intralipid and heparin and of injection of dichloroacetate on myocardial extractions of glucose, lactate and pyruvate were investigated by measurements of concentration differences between arterial and coronary sinus blood. Measurements of arterial blood O_2 saturation, the observation of persistent positive arteriovenous differences in lactate concentration and of coronary sinus [lactate]/[pyruvate] ratios comparable with those of arterial blood indicate that adequate myocardial oxygenation was achieved in these experiments. The observation that elevation of plasma free fatty acid concentrations with intralipid and heparin increases free fatty acid extraction and decreases extractions of glucose, lactate and pyruvate is consistent with the view that free fatty acids may inhibit glucose uptake and pyruvate oxidation by dog heart *in vivo*. This interpretation assumes that intralipid and heparin do not substantially increase coronary blood flow, but it was not possible to obtain evidence on this point. Injection of dichloroacetate in dogs receiving an infusion of intralipid and heparin substantially increased myocardial extractions of glucose, lactate and pyruvate. This observation is consistent with effects of dichloroacetate on glycolysis and pyruvate oxidation in the perfused rat heart. Dichloroacetate also increased myocardial extractions of pyruvate and lactate in the alloxan-diabetic dog in conformity with its effects on pyruvate oxidation in hearts from alloxan-diabetic rats *in vitro*.

Mechanism of action of dichloroacetate

Pyruvate oxidation. In rat diaphragm muscle dichloroacetate only increased glucose or pyruvate oxidation when this was inhibited by alloxan diabetes or by 3-hydroxybutyrate. In hearts from normal rats dichloroacetate produced a small increase in glucose oxidation in perfusions with glucose and insulin. More substantial increases in glucose oxidation were only seen when this was inhibited by alloxan diabetes or acetate or 3-hydroxybutyrate. Dichloroacetate also inhibited oxidation of acetate, 3-hydroxybutyrate and palmitate and this might suggest that dichloroacetate activates oxidation of glucose and pyruvate by inhibiting the oxidation of these other substrates. Stacpoole & Felts (1970) have suggested that dichloroacetate may increase glucose oxidation in diabetic muscle by inhibiting fatty acid oxidation. The changes in metabolite concentrations with dichloroacetate in the present study may not be consistent with this interpretation for the following reasons.

Metabolism of acetate in rat heart increases tissue concentrations of acetyl-CoA, acetylcarnitine, citrate and glutamate and lowers that of aspartate (Randle *et al.*, 1970). If dichloroacetate improves glucose oxidation in acetate perfusions by inhibiting acetate oxidation then it might be expected to reverse the effects of acetate on tissue-metabolite concentrations. Although dichloroacetate lowered citrate concentration it did not lower tissue acetyl-CoA and acetylcarnitine concentrations. Moreover, measurements of specific radioactivities in perfusions with [1-^{14}C]-acetate indicated that a substantial proportion of the accumulated acetyl-CoA was derived from a non-radioactive precursor (presumably pyruvate) in perfusion with dichloroacetate. This suggested that dichloroacetate may directly increase pyruvate oxidation. The observation that dichloroacetate increased tissue concentrations of acetyl-CoA and acetylcarnitine and lowered that of pyruvate in glucose perfusions is consistent with this interpretation. In hearts perfused with glucose and insulin approx. 80% of the O_2 consumed may be utilized by oxidation of glucose, and this presumably explains why dichloroacetate has only small effects on glucose oxidation under these conditions (Randle *et al.*, 1970).

Whitehouse & Randle (1973) observed that dichloroacetate perfusion in rat heart substantially increases the activity of pyruvate dehydrogenase measured in extracts of the tissue. They concluded that this activation may result from the conversion of inactive (phosphorylated) pyruvate dehydrogenase into an active form. In glucose perfusions therefore dichloroacetate may activate pyruvate dehydrogenase and accelerate pyruvate oxidation, leading to accumulations of acetyl-CoA and acetylcarnitine. The increased concentration of acetyl-CoA may result in an increased rate of flow in the tricarboxylate cycle span from acetyl-CoA to glutamate, leading to accumulation of glutamate and consumption of oxaloacetate formed by malate dehydrogenase and

glutamate–aspartate aminotransferase, with concomitant lowering of malate and aspartate concentrations.

Citrate concentration, glycolytic flux and phosphofructokinase activity. The rate of glycolysis was increased by dichloroacetate in rat hearts in acetate perfusions. It is suggested that this is due to lowering of cell citrate concentration with concomitant activation of phosphofructokinase. Stacpoole & Felts (1971) observed that dichloroacetate lowers citrate concentration in hearts and diaphragms from alloxan-diabetic rats and suggested that this may have resulted from inhibition of fatty acid oxidation. Our observations may not be consistent with this interpretation for reasons summarized in the preceding section.

We have been unable to obtain convincing evidence for the mechanism by which dichloroacetate lowers heart citrate concentration in perfusions with acetate and for the failure of dichloroacetate to raise the concentration of citrate in parallel with those of acetyl-CoA and glutamate in perfusions with glucose. One possibility is a deficiency of oxaloacetate as a result of increased incorporation of oxaloacetate into glutamate and impaired conversion of 2-oxoglutarate into oxaloacetate (suggested by the lower concentrations of aspartate and malate). The lowering of heart pyruvate concentration with dichloroacetate might contribute to oxaloacetate deficiency by restricting transamination between glutamate and pyruvate, which appears to be associated with citrate accumulation during acetate metabolism (Randle *et al.*, 1970). Some evidence in support of these possibilities has been obtained in rat hearts perfused with media containing glucose, insulin and 5mM-pyruvate (not shown). Perfusions with pyruvate increased heart citrate concentration and dichloroacetate did not lower citrate concentration with pyruvate. Other possibilities include activation by dichloroacetate of one of the reactions between citrate and glutamate.

Oxidation of acetate, 3-hydroxybutyrate and palmitate. Dichloroacetate inhibits the oxidation of acetate, 3-hydroxybutyrate and palmitate in rat heart and diaphragm muscles. The mechanism of this effect is not known. The common requirement for CoA of pyruvate dehydrogenase, acetyl-CoA synthetase, acyl-CoA synthetases and β-oxoacyl-CoA thiolases suggests as a possible mechanism competition for CoA between pyruvate dehydrogenase, which is activated by dichloroacetate (Whitehouse & Randle, 1973), and other enzymes utilizing coenzyme A. Studies with isolated mitochondria may yield evidence on this mechanism.

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