Effects of Dichloroacetate on Lipid Metabolism in Isolated Rat Liver Cells

ROBERT I. MISBIN

SUMMARY

Administration of dichloroacetate (DCA) to normal rats resulted in a fall in serum glucose and triglycerides and a rise in ketone bodies. Insulin and cholesterol levels were unchanged. The effects of DCA on lipid metabolism were examined in isolated rat hepatocytes. At 10 mM DCA, the incorporation of tritiated water into fatty acids (saponifiable lipids) was inhibited by 33 ± 4% (mean ± SEM, N = 5). No effect on incorporation into cholesterol (measured as nonsaponifiable lipids) was observed. DCA inhibited the incorporation of ^{14}C-glucose into lipid but had no effect on glucose oxidation. Fatty acid oxidation was increased by 76 ± 7% (mean ± SEM, N = 6). However, DCA had no effect on the recovery of newly synthesized lipid. Thus, inhibition of tritiated water incorporation into fatty acids represents decreased synthesis rather than increased turnover. DCA did not affect the incorporation of ^{14}C-palmitate into triglycerides or phospholipids. Cell viability, as assessed by incorporation of ^{3}H-isoleucine into protein and trypan blue exclusion, was not affected by DCA. These results suggest that DCA lowers serum triglycerides through inhibition of fatty acid synthesis and stimulation of fatty acid oxidation by liver. DIABETES 28:265–271, April 1979.

Dichloroacetate (DCA) lowers blood glucose in laboratory animals[1–4] and ameliorates lactic acidosis induced experimentally with phenformin.[5,6] DCA stimulates the utilization of glucose, lactate, and pyruvate by extrahepatic tissues at the expense of fatty acid oxidation.[7,8] The fall in blood concentrations of alanine and lactate in animals treated with DCA leads to a decrease in glucose production by the liver due to lack of substrate.[3] In addition, DCA directly inhibits hepatic gluconeogenesis even when substrates are present in excess.[9] Some of the effects of DCA on carbohydrate metabolism are attributable to its activation of the pyruvate dehydrogenase enzyme complex in heart and skeletal muscle.[10] This enzyme is inactivated by phosphorylation. DCA appears to "lock" the enzyme in its active form by inhibiting pyruvate dehydrogenase kinase.[11]

The results of the first clinical trial of DCA for the treatment of diabetes were recently reported by Stacpoole et al.[12] Consistent with the animal work discussed above, DCA caused a rapid decline in lactate and alanine followed by a fall in plasma glucose. The effects of DCA on plasma triglyceride levels were particularly impressive. All seven patients showed a fall in plasma triglycerides that ranged from 19–67%. All but one showed a fall in plasma cholesterol. A rise in plasma beta-hydroxybutyrate was also noted. The authors speculated that the fall in triglycerides could be a consequence of decreased substrate (glucose, lactate) availability, direct inhibition of hepatic lipogenesis, or stimulation of triglyceride oxidation. However, there were insufficient data with which to assess the relative importance of these possible mechanisms.

The present study was undertaken to help elucidate the effects of DCA on hepatic lipid metabolism. Oral administration of DCA to normal rats resulted in decreased serum levels of glucose and triglycerides but no change in cholesterol. DCA inhibited fatty acid synthesis by liver cells in vitro and stimulated fatty acid oxidation. No effect on cholesterol (measured as nonsaponifiable lipid) synthesis was observed. The increase in fatty acid oxidation in liver is of particular interest because DCA is known to decrease fatty acid oxidation in muscle.[7,8] Thus, the effects of DCA on lipid metabolism are complex. The fall in serum triglycerides in animals treated with DCA appears to be a consequence of decreased synthesis and increased oxidation of fatty acids in liver.

MATERIALS AND METHODS

Studies in vivo. Male Sprague-Dawley rats weighing approximately 150 g were obtained from the breeding farm at the University of Florida. Rats were fed Purina rat chow ad libitum until they achieved a weight of 260–320 g. The rats

From the Department of Medicine, University of Florida School of Medicine, Gainesville, Florida 32610.
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TABLE 1
Effects of DCA in vivo

	DCA	Control	P
Glucose (mM)	5.7 ± 0.3	7.3 ± 0.2	<0.005
Triglyceride (mg/dl)	38 ± 4	53 ± 4	<0.05
Cholesterol (mg/dl)	61 ± 5	62 ± 3	NS
FFAs (mM)	0.550 ± 0.033	0.440 ± 0.035	<0.05
Betahydroxybutyrate (mM)	1.02 ± 0.12	0.42 ± 0.06	<0.001
Acetoacetate (mM)	0.77 ± 0.09	0.25 ± 0.03	<0.001
Lactate (mM)	1.23 ± 0.08	1.42 ± 0.07	<0.10
Alanine (mM)	0.32 ± 0.04	0.50 ± 0.03	<0.01
Insulin (μU/ml)	5.2 ± 1.0	8.0 ± 2.3	NS

DCA, dichloroacetate; FFAs, free fatty acids.

Drinking water was enriched with 20 mM DCA or 20 mM NaCl (control). Rats consumed approximately 50 ml/day. Serum specimens were obtained after 14 days of treatment.

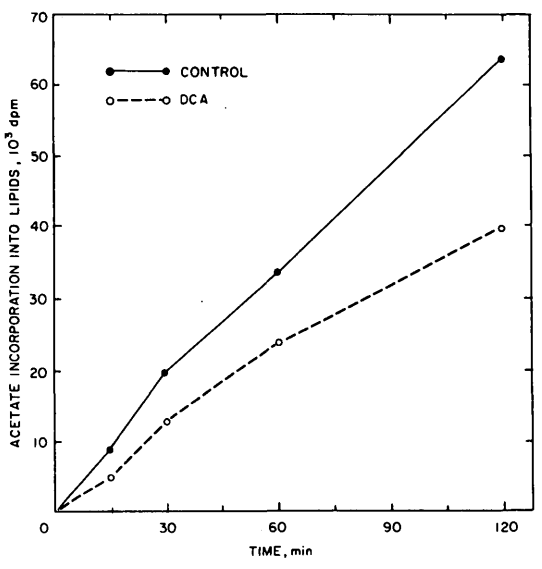
were then divided into two groups of approximately equal weight. The experimental group received 20 mM sodium DCA in its drinking water. The control group received 20 mM sodium chloride. At the end of 14 days, rats were deprived of food for 16–18 h and anesthetized by an intraperitoneal injection of 15 mg of pentobarbital. Eight milliliters of blood was obtained from the abdominal aorta. Sera were stored at −20°C until assayed for insulin, free fatty acids (FFAs), glucose, triglycerides, and cholesterol. Samples obtained for measurement of lactate, alanine, and ketone bodies were deproteinized with an equal volume of 2 N perchloric acid before storage.

Preparation of hepatocytes. Hepatocytes were prepared according to a modification of the collagenase method described by Feldhoff et al.[13] Male Sprague-Dawley rats were maintained on Purina rat chow until they weighed 175–210 g. Rats were allowed to eat ad libitum until they were anesthetized by an intraperitoneal injection of 10 mg of pentobarbital. The portal vein was cannulated and perfused without recirculation at about 15 ml/min with calcium-free Krebs-Ringers bicarbonate buffer (pH 7.4) containing 12.5 mM glucose, 5 mM sodium pyruvate, 5 mM monosodium glutamate, and 10–15% fresh steer erythrocytes that had been washed three times with saline. The perfusate was maintained at 37°C and equilibrated with humidified oxygen/carbon dioxide (95:5%). After 3 min, the system was set to recirculate with 150 ml of the same buffer containing 20 mg of collagenase (Worthington Biochemical, Freehold, New Jersey, Type II). After 20 min, the liver was cut into several pieces and resuspended in 100 ml of fresh buffer containing 1% bovine serum albumin (Sigma Fraction V) and no erythrocytes. The suspension was divided among three 250-ml plastic Erlenmeyer flasks, gassed with oxygen/carbon dioxide, stoppered tightly, and incubated at 37°C in a shaker bath (Forma Scientific, Marietta, Ohio) at 140 cycles/min for 20 min. The suspension was then filtered through nylon gauze into 15-ml conical plastic centrifuge tubes. The hepatocytes were collected by gentle centrifugation for about 30 s in a clinical centrifuge. The supernatant (containing red blood cells and debris) was removed by aspiration. The cells were washed three times by resuspension with 15 vol of fresh oxygenated buffer now containing 2.59 mM $CaCl_2$. The washed cells were then resuspended in 15 vol of buffer. Viability was determined by trypan blue exclusion; it was always greater than 90% and usually greater than 95%. Several experiments were performed to assess the release of preformed cellular lipids into the medium. In these experiments, 25 μCi of ^{14}C-acetate was added to the 100 ml of calcium-free medium during the period when the cells were dispersed by shaking. The cells were then filtered through nylon gauze as usual, but were washed five instead of three times so as to effectively remove unincorporated ^{14}C-acetate.

Incubation of hepatocytes. One milliliter of 1:15 suspension (about 2 million cells) was added to 25-ml flasks containing 2 ml of oxygenated Krebs-Ringers bicarbonate to which was added 2 mCi of ^3H_2O (100 mCi/g), 0.5 μCi ^{14}C-glucose (313 mCi/mmol), 2 μCi ^{14}C-acetate (54 mCi/mmol), 10 μCi ^{3}H-isoleucine (100 Ci/mmol), or 0.4 μCi ^{14}C-palmitate (613 mCi/mmol) (all ^{14}C reagents were uniformly labeled and were obtained from New England Nuclear, Boston, Massachusetts) and sodium dichloroacetate (Ciba-Geigy, Ardsley, New York) or an equimolar quantity of NaCl. Except where otherwise specified, the concentration of DCA was 10 mM. The flasks were gassed with oxygen:carbon dioxide (95:5%), tightly stoppered, and incubated for 60 min (unless otherwise specified) with constant shaking, at 37°C. The reaction was stopped by plunging the flasks into ice water, by the addition of 0.6 ml concentrated perchloric acid (in experiments where generation of $^{14}CO_2$ was measured), or by the addition of 3 ml of 10% trichoroacetic acid (in experiments where incorporation of ^{3}H-isoleucine into protein was measured).

Analytic methods. Incorporation of radioactive precursors into lipids was measured by extraction of the cellular pellet (obtained by vigorous centrifugation) with 2:1 chloroform-methanol according to the method of Folch.[14] The extract was washed three times with "upper phase" to remove unincorporated lipid precursors. This procedure kept the level of contamination to less than 3% of the extracted radioactivity. Controls were performed for each experiment so that the data could be corrected for the contamination. In several experiments, the residue of the chloroform-methanol extract

FIGURE 1. Effect of dichloroacetate on acetate incorporation into lipid. Cells were incubated in the presence of 2 μCi ^{14}C-acetate and 10 mM dichloroacetate (DCA). Data represent the mean of triplicates from one of three experiments and are expressed per 2 million hepatocytes.



was saponified by addition of 2 ml of 5N NaOH, 3 ml of absolute ethanol, and heating at 80°C for 3 h. Two milliliters of water was added and the cholesterol-rich fraction (nonsaponifiable lipid) was extracted three times with 5 ml of petroleum ether. The fatty acid fraction (saponifiable lipid) was extracted with petroleum ether after acidification with 1.25 ml of 10 M H_2SO_4. The organic extracts were transferred to scintillation vials, evaporated to dryness, and counted in a Packard Liquid Scintillation Counter using 10 ml of 3a70B counting cocktail (Research Products International, Elk Grove Village, Illinois). Data were corrected for quenching by standard methods and expressed as disintegrations per minute. In order to determine contamination of the nonsaponifiable fraction with saponifiable lipids, a standard containing ^{14}C-palmitate was carried through the saponification and extraction procedure. A quantity of 1.7–1.8% of the total counts appeared in the nonsaponifiable fraction. The data were corrected for this contamination. Total recovery of standard solutions of ^{14}C-cholesterol and ^{14}C-tripalmitin were each greater than 95%. In several experiments, lipid fractions were isolated by thin-layer chromatography on silica gel plates using a solvent system containing petroleum ether:ethyl ether:acetic acid (80:20:1). To determine the generation of $^{14}CO_2$, 0.2 ml of hyamine hydroxide (New England Nuclear) was injected into plastic cups suspended in the incubation flask after the reaction had been stopped by addition of 0.6 ml of concentrated perchloric acid. After agitation of the flasks at room temperature for 30 min, the cups were transferred to scintillation vials and counted with 10 ml of scintillation fluid. Incorporation of ^{3}H-isoleucine into protein was measured by precipitation with 3 ml of 10% trichloroacetic acid. The precipitate was washed three times with 6 ml of 5% trichloroacetic acid, dissolved in 0.5 ml of 1 mM HCl, and counted. The specific activity values of ^3H_2O, ^{14}C-glucose, and ^{14}C-palmitate were determined by measuring the radioactivity of an aliquot of medium before and after the incubation. The concentration of H_2O in the medium was found to be 54.5 M from the difference between the net weight and the dry weight. Free fatty acids were assayed colorimetrically.[15] Glucose, lactate, pyruvate, betahydroxybutyrate, and acetoacetate were assayed enzymatically.[16] Serum cholesterol and triglycerides were assayed by standard automated methods.[17,18] DNA was assayed by the methods of Burton and Schneider.[19]

FIGURE 2. Dose–response relationship—acetate incorporation into lipids. Data represent mean ± SEM of three experiments. Flasks contained the indicated concentration of dichloroacetate or an equimolar concentration of NaCl in controls. Cells were incubated for 60 min. Data are expressed per 2 million hepatocytes.

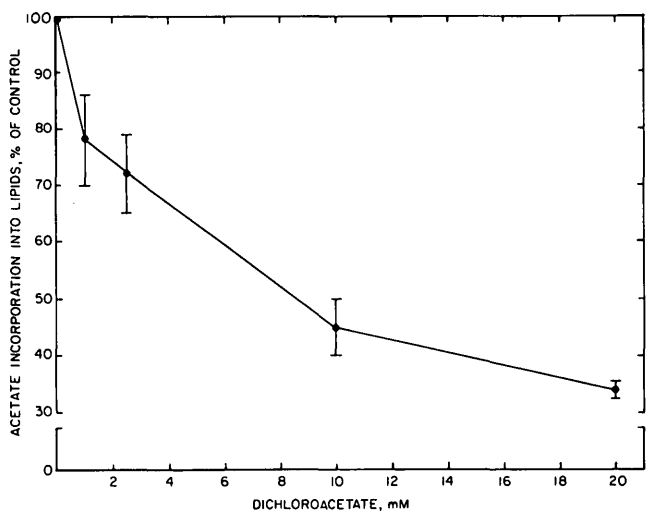


TABLE 2
Acetate incorporation into total lipids and carbon dioxide

	Total lipids	Carbon dioxide
Control (dpm, 10^3)	32.5 ± 8.2	8.1 ± 2.6
DCA (dpm, 10^3)	15.3 ± 4.6	5.5 ± 2.0
$\frac{\text{DCA}}{\text{Control}}$ (%)	47.5 ± 5.3	65.0 ± 7.2
Number of experiments	7	6
P	<0.001	<0.01

DCA, dichloroacetate.

Hepatocytes were incubated for 60 min in the presence of 2 μCi ^{14}C-acetate and 10 mM DCA. In control tubes, 10 mM NaCl was added instead of DCA. Data are expressed per 2×10^6 hepatocytes and are mean ± SEM for the specified numbers of observations.

Calculations. The incorporation of ^3H_2O, ^{14}C-glucose, and ^{14}C-palmitate into lipid and/or carbon dioxide was calculated from the following formula:

$$\text{Incorporation} = \frac{\text{Radioactivity in lipid or } CO_2}{\text{Specific activity of precursor}}$$

This calculation assumes that the specific activity is constant throughout the experiment and reflects the intracellular specific activity. With respect to glucose, this assumption would not be valid if there were extensive recycling. Therefore, in several experiments, ^{14}C-glucose was separated from charged intermediates by ion exchange chromatography after 60 min of incubation. The decrease in the radioactivity of the glucose fraction was less than 5% and was not affected by 10 mM DCA. In experiments using ^{14}C-acetate and ^{3}H-isoleucine, the specific activity of the precursor was not measured. Results from these experiments are expressed as disintegrations per minute.

Data were expressed per 2 million cells, which is equal to 2.25 ± 0.24 mg dry wt or 12.5 ± 1.8 μg DNA (mean ± SEM of five determinations). Statistical analysis was done by Student's *t* test.

RESULTS

Effects of DCA in vivo. Rats that had DCA added to their drinking water had significantly lower levels of serum glucose and triglycerides than control rats (Table 1). Serum FFAs, betahydroxybutyrate, and acetoacetate were elevated in the DCA-treated rats, whereas alanine and lactate levels were depressed. Insulin and cholesterol concentrations were not affected. The changes associated with DCA could not be attributed to poor food intake. Rats gained an average of 10% of their initial body weight during the 14-day study regardless of whether or not they received DCA.

Effects of DCA on hepatocytes. As shown in Figure 1, hepatocytes incorporated ^{14}C-acetate into lipids for 2 h. DCA caused a marked inhibition of acetate incorporation that was evident as early as 15 min. Inhibition of acetate

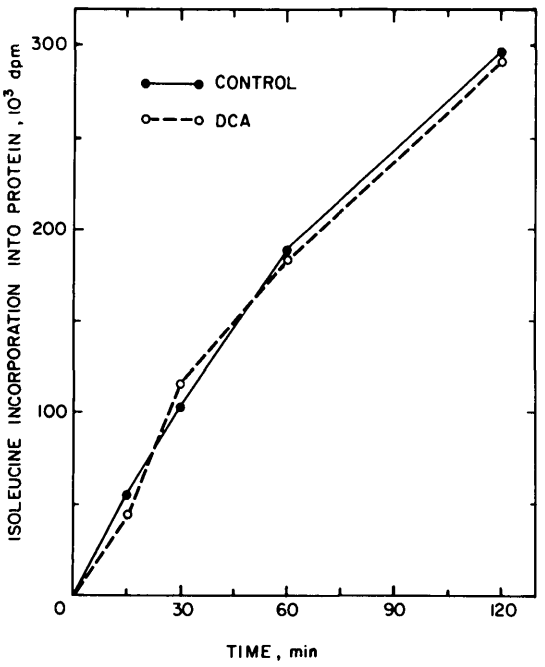


FIGURE 3. Effect of dichloroacetate on protein synthesis. Cells were incubated in the presence of 10 mM dichloroacetate or 10 mM NaCl, 10 μCi ^{3}H-isoleucine, and 25 μg/ml unlabeled Asp, Glu, Leu, Pro, Tyr, Asn, Phe, Try, Gly, Arg, Cys, Meth, Thr, Val, Ala, His, Lys, and Ser. Data represent the mean of triplicates from one of three experiments and are expressed per 2 million hepatocytes.

incorporation into lipid was seen at as low a concentration as 1 mM DCA (Figure 2). DCA also inhibited acetate oxidation but to a lesser extent than incorporation into lipid (Table 2). DCA does not appear to have a generalized inhibitory effect on biosynthetic processes. As shown in Figure 3, incorporation of ^{3}H-isoleucine into protein was unaffected by DCA. Also, DCA did not affect cell viability as measured by trypan blue exclusion.

Dietschy and McGarry[20] have pointed out that incorporation of ^{14}C-acetate into lipid does not necessarily reflect biosynthesis because of dilution of the label with various intracellular pools of unlabeled acetate. Studies were therefore undertaken to determine if DCA affects lipid synthesis from ^3H_2O. As shown in Table 3, lipid synthesis was inhibited 33% in the presence of DCA. This effect was due totally to inhibition of fatty acid (saponifiable lipid) synthesis. No effect on the cholesterol-rich fraction (nonsaponifiable lipid) was detected. Since the nonsaponifiable lipid accounted for only about 5% of the total and was not affected by DCA, further studies were done without saponification. Experiments were performed to determine if DCA affected the incorporation of free fatty acids into triglycerides and phospholipids. As shown in Table 4, no effect was observed. DCA inhibited lipid synthesis from ^{14}C-glucose to the same extent as from ^3H_2O (Table 5). The concentration of glucose in the incubation medium remained relatively constant during the incubation (Table 6). Changes in lactate and pyruvate were also the same with or without DCA. Thus, inhibition of lipid synthesis by DCA was not due to changes in the concentration of lipid precursors. When expressed in terms of acetyl group incorporation into lipids, glucose accounted for about 16% of total acetyl incorporation, whether or not DCA was present (Table 5). With a similar concentration of glucose in the medium, Brunengraber observed a value of about 17% in perfused liver.[21]

As shown in Table 7, DCA had no effect on carbon dioxide generation from glucose, but markedly enhanced carbon dioxide generation from fatty acids. The possibility was therefore considered that the decreased incorporation of ^3H_2O and ^{14}C-glucose into lipid might be a reflection of increased lipid turnover rather than decreased synthesis. A series of experiments was performed to determine if incubation of cells in the presence of DCA affected the recovery of newly synthesized lipid. The details of the experiment are given in the legend to Figure 4 and under Methods. Approximately 85% of the newly synthesized lipid remained within the hepatocytes during 60 min of incubation. Approximately 12% was released into the medium. Similar values have been reported in perfused liver.[22,23] Neither the total recovery of radioactivity nor the distribution of labeled lipid between cell and medium was affected by DCA. DCA did cause a 21 ± 4.3% increase (mean ± SEM, N = 5, $P < 0.01$) in the production of $^{14}CO_2$; however, the total amount of radioactivity recovered as CO_2 was small. It was concluded from these experiments that decreased incorporation of ^3H_2O and ^{14}C-glucose into lipid in the presence of DCA is not attributable to increased lipid oxidation.

DISCUSSION

According to Pilkis et al.,[24] the effects of hormones on gluconeogenesis in isolated rat hepatocytes are similar to those observed in perfused rat liver. The use of isolated liver cells, however, is more convenient than perfused liver because

TABLE 3
Incorporation of ^3H_2O into lipid classes

	Total lipids (nmol)	Saponifiable (nmol)	Nonsaponifiable (nmol)
Control	169 ± 32	161 ± 29	7.3 ± 2.4
DCA	120 ± 29	112 ± 25	8.2 ± 3.2
$\frac{DCA}{Control}$ (%)	69 ± 4	67 ± 4	102 ± 10
P	<0.005	<0.005	NS

DCA, dichloroacetate.

Hepatocytes were incubated for 60 min in the presence of 2 mCi ^3H_2O.

Data are mean ± SEM of five experiments and are expressed per 2 × 10^6 hepatocytes.

TABLE 4
Incorporation of ^{14}C-palmitate into glycerides

	Phospholipids (nmol)	Triglycerides (nmol)
Control	43 ± 11	103 ± 14
DCA	44 ± 4	103 ± 16
$\frac{DCA}{Control}$ (%)	106 ± 4	103 ± 2
P	NS	NS

DCA, dichloroacetate.

Hepatocytes were incubated for 60 min in the presence of 0.4 μCi ^{14}C-palmitate. Lipids were extracted as usual and separated into classes by thin layer chromatography. 90–95% of palmitate incorporated into extractable lipids was accounted for as triglyceride and phospholipid. Data are mean ± SEM of four experiments and are expressed per 2 × 10^6 hepatocytes.

it allows for several metabolic processes to be examined under different experimental conditions in a homogeneous population of cells. Using a method of cell isolation similar to the one employed in this study, Feldhoff et al.[13] demonstrated that hepatocytes synthesized and secreted albumin at rates comparable with perfused liver, while maintaining viability for as long as 3 h. Table 8 shows a comparison of data on lipid metabolism in isolated hepatocytes to what has been observed in perfused rat liver and in vivo. The results reported here are similar to what has been observed in other systems, thus validating the use of isolated hepatocytes for studies of lipid metabolism.

The fall in serum triglycerides, glucose, alanine, and lactate and the rise in serum ketone bodies in rats on DCA are similar to the effects reported by Stacpoole et al. in diabetic patients.[12] Examination of the mechanism whereby DCA lowers triglyceride levels in rats may be relevant to its effects in man. Table 4 shows that DCA did not affect the incorporation of FFAs into triglyceride. It is therefore reasonable to assume that DCA lowers serum triglycerides by decreasing the amount of FFAs available in the liver for incorporation into triglyceride or by increasing triglyceride clearance by extrahepatic tissues. Brunengraber has shown that the rate of fatty acid synthesis in perfused rat liver is in part a function of the glucose concentration in the perfusate.[21] The fall in serum glucose in rats treated with DCA would of itself be expected to decrease fatty acid synthesis by liver. The present study demonstrates that DCA directly inhibits fatty acid synthesis by liver under conditions where substrate availability is not a limiting factor. DCA also stimulated fatty acid oxidation. Thus, we have identified two direct actions on the liver that would reduce the amount of fatty acid available to be incorporated into triglyceride. In rats, DCA does not appear to affect serum cholesterol levels or cholesterol synthesis in liver. This study provides no explanation for the small, but statistically significant, fall in plasma cholesterol observed in diabetic patients on DCA.[12]

Stimulation of fatty acid oxidation by DCA in liver cells was an unexpected finding. It had previously been reported by different laboratories that DCA inhibited fatty acid oxidation in muscle.[7,8] The small rise in serum FFA levels in rats treated with DCA in the present study is consistent with these reports because the effects of DCA on fatty acid metabolism in liver could only lower the serum levels. The dramatic rise in serum ketone bodies in rats treated with DCA is also best explained by decreased uptake in extrahepatic tissues,[8,25] because we observed no direct effects on hepatic ketogenesis. The production of ketone bodies by liver cells was 89 ± 42 mM/2 × 10^6 cells/h in the presence of 10 mM DCA and 78 ± 41 in its absence (mean ± SEM, N = 5). It must be emphasized that the present study was undertaken to study fatty acid synthesis not ketogenesis. Animals were therefore fed ad libitum, and incubations performed with a medium rich in carbohydrate and poor in fatty acids. Using liver cells from starved rats, Stacpoole found a much higher rate of ketone body formation (Table 8). He also found that DCA stimulated ketone production in the absence of added substrates (presumable fatty acid in the medium was the substrate). It has also been reported that DCA increases ketone levels in rats with lactic acidosis[5] but decreases ketone levels in rats with ketoacidosis.[25] It appears that DCA increases ketone production from pyruvate and lactate but decreases ketone production from fatty acids.

The inhibition of fatty acid synthesis by DCA observed in the present study is supported by recent work of Stansbie and Sherriff.[33] These investigators found that fatty acid synthesis was depressed in liver and adipose tissue taken from rats treated with DCA. In contrast, Crabb et al. reported that DCA stimulated fatty acid synthesis in hepatocytes at

TABLE 6
Initial and final concentrations of substrates

	Initial	Final	
		DCA	Control
Glucose (mM)	12.9 ± 0.5	12.6 ± 0.6	12.4 ± 0.6
Pyruvate (mM)	4.24 ± 0.10	2.79 ± 0.25	2.52 ± 0.25
Lactate (mM)	0.22 ± 0.02	0.63 ± 0.07	0.74 ± 0.08
Pyruvate + lactate	4.45 ± 0.10	3.42 ± 0.73	3.25 ± 0.30
FFAs (mM)	0.141 ± 0.014	0.159 ± 0.011	0.162 ± 0.006

DCA, dichloroacetate; FFAs, free fatty acids.
Initial concentrations are before incubation. Final concentrations are after 60 min in the presence and absence of 10 mM DCA. Data are mean ± SEM of six experiments. No differences between DCA and control in final concentrations were statistically significant.

TABLE 5
Incorporation of ^3H_2O and ^{14}C-glucose into total lipid

	^3H_2O (nmol)	^{14}C-glucose (nmol)	Glucose incorporation (percent total acetyl incorporation)
Control	166 ± 24	10.5 ± 2.7	15.3 ± 3.3
DCA	116 ± 20	7.2 ± 1.7	16.0 ± 3.3
$\frac{DCA}{Control}$ (%)	67 ± 3	71 ± 4	109 ± 6
Number of experiments	8	5	4
P	<0.001	<0.005	NS

DCA, dichloroacetate.
Hepatocytes were incubated for 60 min in the presence of 2 mCi ^3H_2O or 0.5 μCi glucose. The calculation of acetyl incorporation into lipid assumes that 1.15 nmol of acetyl group is incorporated per nanomole of ^3H_2O.[21,31]

TABLE 7
$^{14}CO_2$ production from glucose and fatty acids

	Glucose (nmol)	Fatty acids (nmol)
Control	33.8 ± 13.3	2.9 ± 0.4
DCA	32.3 ± 12.7	5.1 ± 0.5
$\frac{DCA}{Control}$ (%)	100 ± 8	176 ± 7
P	NS	<0.001

DCA, dichloroacetate.
Hepatocytes were incubated for 60 min in the presence of 0.5 μCi ^{14}C-glucose or 0.4 μCi ^{14}C-palmitate. The concentrations of glucose and fatty acid in the incubation medium were 12.9 mM and 0.14 mM, respectively. Data are mean ± SEM of six experiments.

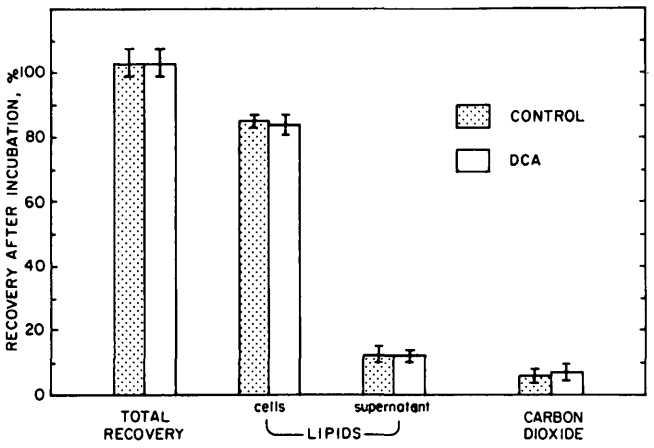


FIGURE 4. Effects of dichloroacetate on fate of preformed lipids. Hepatocytes were incubated with ^{14}C-acetate during the isolation procedure. Cells were washed extensively and incubated for 60 min in the presence and absence of dichloroacetate. Radioactive lipids were extracted from the cellular pellet and cell-free supernatant. $^{14}CO_2$ was collected in hyamine. Radioactivity is expressed as percent of ^{14}C lipids that were present in the cells before incubation. The ^{14}C lipid content of the cells was 1354 ± 536 dpm/2 × 10^6 cells.

a concentration of 1 mM but had no effect at 10 mM.[34] Their results regarding the effects of DCA on gluconeogenesis from alanine are also at variance with those of Stacpoole.[9] Crabb and Harris have suggested that conversion of DCA to glyoxylate could account for some of its apparent pharmacologic activity.[35] Perhaps factors influencing the metabolism of DCA itself account for these disparate effects observed in vitro.

In summary, the present study has demonstrated that DCA inhibits fatty acid synthesis and stimulates fatty acid oxidation in rat hepatocytes. These data suggest that inhibition of triglyceride output from liver accounts for the decreased level of serum triglycerides in rats treated with DCA.

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TABLE 8
Comparison of isolated hepatocytes with other systems

	Lipid synthesis					
	Saponifiable			Incorporation of fatty acids into:		
	Acetyl group	Acetyl group from glucose, percent of total	Nonsaponifiable acetyl group	CO_2	Glyceride	Ketone production
Isolated hepatocytes						
Present study	83	16	4.3	1.2	67	35
Other studies	70*	—	9.6*	—		86†
Perfused liver	170‡	17‡	5.7‡	1.0‖	58§	31‡
	93§	19§	8.0§	—		34¶
In vivo	58#	—	8.1#	—		
	62**					
	178‡		4.4‡			

* Chow, meal fed, 20 mM glucose in medium.[26]
† 18-h fasted, no substrate added to medium.[9]
‡ High carbohydrate "meal fed"—15 mM glucose in medium except 25 mM glucose for ketones.[21]
§ Chow ad libitum, 15 mM glucose and 5 mM lactate in medium.[23]
‖ Low-fat diet, medium supplemented with oleate.[27]
¶ Chow ad libitum, medium supplemented with oleate.[28]
# Chow ad libitum.[21]
** Chow ad libitum.[29]

Data are expressed as nanomoles per gram dry weight per hour. The following conversion factors were used: 200 g rat = 9.5 g liver wet wt;[30] 1 g wet wt = 0.31 g dry wt;[21] 2 × 10^6 hepatocytes = 2.25 mg dry wt. To express data as micromoles of acetyl group, the incorporation of ^3H_2O into saponifiable and nonsaponifiable lipids was multiplied by 1.15 and 1.31, respectively.[21,31,32]

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