Beta-adrenoceptor blocking drugs influence aggregation of blood platelets in concentrations 0.001 mM and more [14–16]. In our experiments concentrations required for affecting ecto-ATPase activity were 100-fold higher. These data suggest other mechanisms are responsible for the action of beta-adrenoceptor blocking drugs on the blood platelet aggregability than alterations in the platelet ecto-ATPase activity. Such mechanisms may be related to the availability of aggregation receptors, calcium availability and to the intraplatelet cyclic nucleotide levels. Further studies with platelets for elucidation of this problem are under way.

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Dichloroacetate tissue concentrations and its relationship to hypolactatemia and pyruvate dehydrogenase activation

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Dichloroacetate (DCA) activates the pyruvate dehydrogenase complex (EC 1.2.4.1) (PDH) by inhibition of PDH kinase [1, 2]. Inhibition of this regulatory enzyme allows unopposed activation of PDH by PDH phosphatase. Administration of the drug to humans [3, 4] or laboratory animals [5, 6] causes prompt lowering of blood glucose by increased utilization of gluconeogenic precursors in peripheral tissues [7]. DCA lowers blood lactate, and with chronic administration this effect persists for days following discontinuance of the drug despite the relatively short plasma half-life of DCA [3, 4, 8]. Basal PDH activity remains increased for 12–24 hr following chronic DCA administration and total PDH activity remains increased for 24–48 hr [9]. The prolonged effects of DCA are not due to its metabolites, oxylate and glyoxylate, because they do not activate PDH *in vitro* [9]. The purpose of this study was to determine tissue concentrations of DCA and how they relate to the prolonged effects of the drug.

Adult Sprague–Dawley rats, 200–250 g, were used throughout the study and had free access to water and Purina rat chow.

DCA was administered by gastric intubation, 100 mg/kg in a solution of 200 mg/ml of saline. For single dose experiments, DCA was given and three animals were killed at 1, 3, 6, 12 and 24 hr following administration. In chronic experiments, 100 mg/kg of DCA was given as a single daily dose by gastric intubation for 7 days. Three animals were then killed at 3, 6, 12, 24, 48 and 72 hr after the final dose. The animals were sacrificed under sodium pentobarbital anesthesia and exsanguinated via the abdominal aorta. Livers were excised rapidly and freeze-clamped in liquid nitrogen and stored at −70° until assayed. In some animals, muscle tissue was similarly obtained and stored.

PDH was assayed both at basal activity and following *in vitro* activation by methods previously reported [9, 10]. The percent activation was calculated as basal PDH/total PDH × 100. To determine DCA tissue concentrations, the tissue was homogenized in 10 vol. of distilled water for 30 sec in an Ultraturrax tissuemizer at full speed. It was then sonified for three 30-sec periods using a Branson sonifier. The homogenate was then centrifuged at 5000 *g* for 15 min. The concentration of DCA in the supernatant fraction was assayed using electron capture gas chromatography by the method of Sanello [11] as modified by Wells *et al.* [3]. All *in vitro* experiments were performed in duplicate.

DCA was obtained from the Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. [^{14}C-1]Pyruvate was from the New England Nuclear Corp. (Boston, MA) and all other chemicals were from the Sigma Chemical Co. (St. Louis, MO).

Statistical significance between control and treated groups was determined by Student's *t*-test.

Following a single dose of 100 mg/kg DCA, tissue concentrations of DCA increased to a maximum concentration

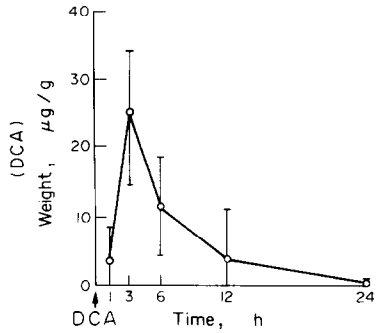

Fig. 1. Liver DCA concentrations following a single dose of DCA. Rats were given 100 mg/kg DCA intragastrically as a single dose and killed following the dose, as described in the text. Each value is the mean of three animals ± S.E.M.

of 25.0 μg/g weight at 3 hr (Fig. 1). Following a 7-day administration of 100 mg per kg per day of DCA, PDH activation was maximum at 3 hr and returned to basal activity 24 hr following the final dose (Fig. 2). Liver DCA concentrations followed a similar pattern, with a maximum concentration of 66.1 μg/g weight at 3 hr and a slow elimination over 72 hr (Fig. 2). The tissue half-life of DCA was calculated to be 9.74 hr using the least squares method for determining the best fit linear regression after logarithmic transformation of concentrations ($R = 0.996$).

In a separate experiment, liver and muscle were obtained 4 hr after the last of seven daily 100 mg/kg DCA doses. Both tissues showed very similar drug concentrations, with muscle somewhat less than liver. PDH activation was increased significantly in both muscle and liver (Table 1).

Tissue concentrations of DCA correlate better with its hypolactatemic effect than with PDH activation. We previously showed that DCA causes significant lowering of blood lactate at 3 hr following a single dose and that with chronic administration blood lactate remains lowered until 48 hr following the final dose [9]. There was no significant PDH activation by DCA with a single dose [9], and with chronic dosing the PDH activation returned to basal activity at 24 hr following the final dose (Fig. 2). DCA tissue concentration, however, peaked at 3 hr following a single dose (Fig. 1) and following chronic administration the drug was slowly eliminated over 72 hr (Fig. 2).

DCA has been shown *in vitro* to have similar effects on all tissues studied [2], and this study shows similar distribution of the drug in muscle and liver tissues. The lowering of blood lactate concentrations is probably the result of reduced gluconeogenic precursors secondary to increased peripheral utilization of pyruvate and lactate [7].

The plasma half-life of DCA varies considerably among species. In humans it is approximately 0.5 hr [3, 8], whereas in rats it is approximately 3 hr [8]. In dogs it is as long as 20 hr [8]. In all species, however, the metabolic effects are prolonged, lasting for days in humans [3, 4] and rats [9]

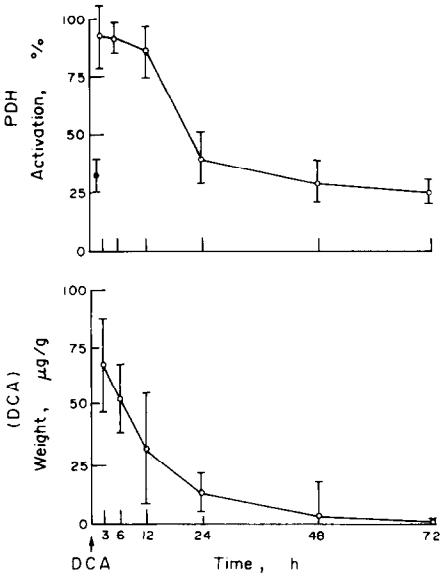

Fig. 2. Liver DCA concentrations and liver PDH percent activation following chronic DCA administration. Rats were given 100 mg per kg per day as a single intragastric dose for 7 days and then killed following the final dose, as described in the text. Each value is the mean of three animals ± S.E.M. Symbols: (●) control; (○) DCA-treated; and (*) $P < 0.05$.

and as long as a week in dogs [6]. This is seen both in normal fed animals as well as diabetic animals. In this study, the half-life in hepatic tissue was 9.74 hr. This suggests that the drug is concentrated within the tissues and that plasma levels inadequately reflect concentrations at the cellular level.

Total radioactivity from ^{14}C-labeled DCA has a longer plasma half-life of 21–36 hr in the rat than DCA concentrations determined by gas chromatography, indicating extensive metabolism and slow elimination [8]. DCA is metabolized to glyoxylate and oxylate [12] which are inhibitory of PDH *in vitro* [9]. Thus, the prolonged hypolactatemic effect was probably related to the accumulation of the drug in the tissues and its slow elimination and not to active metabolites.

Chronic DCA administration increases total PDH activity in addition to activating the PDH complex [9]. This effect was not blocked by protein synthesis inhibitors and persisted for 24–48 hr following the final dose, whereas the activation effect lasts only 12–24 hr. The increased total PDH activity paralleled the prolonged hypolactatemia. Because DCA tissue concentrations persisted for this period, its accumulation may have contributed to the increased total PDH activity and thus the prolonged hypolactatemia.

Table 1. Tissue concentration of DCA and its effect on PDH activation*

Tissue	PDH (% activation)		Tissue DCA (μg/g weight)
	Control	DCA-treated	
Liver	28.5 ± 1.7	87.9 ± 4.3†	88.9 ± 6.1
Muscle	29.2 ± 6.8	62.2 ± 8.4‡	79.6 ± 3.9

* Rats were given 100 mg per kg per day DCA by gastric intubation for 7 days and killed 4 hr after the final dose. Units represent the mean of five animals ± S.E.M.

† Significantly different from control ($P < 0.001$).

‡ Significantly different from control ($P < 0.02$).

In summary, following a single oral dose of DCA to laboratory rats, peak hepatic tissue DCA concentration occurred at 3 hr. When given chronically for 7 days, DCA caused activation of the pyruvate dehydrogenase complex which returned to basal activity 24 hr following the final dose. Hepatic tissue DCA concentrations were maximally increased at 3 hr following the final dose, and the drug was eliminated slowly over 72 hr with a half-life of 9.74 hr. Liver and muscle showed similar DCA tissue concentrations following chronic administration.

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Inhibition of carrageenin-induced rat footpad edema by systemic treatment with prostaglandins of the E series

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One of the most widely used models for evaluation of anti-inflammatory agents is carrageenin-induced edema in the rat footpad [1]. This reaction consists of three distinct phases of mediator-induced vascular permeability changes. The initial phase, during the first 30 min after injection, results from the release of histamine and serotonin from host cells and is inhibited by anti-histamines [2]. The second phase, between 1 and 2.5 hr after injection, is attributed to the action of bradykinin since pretreatment of rats with cellulose sulfate (which lowers plasma kininogen levels) reduces edema. The third phase of persistent edema is complement-dependent and has been attributed to the local production of prostaglandins, especially of the E series, by inflammatory cells [3–6]. This phase of edema formation is inhibited by indomethacin and aspirin, and is potentiated by the local injection of vasodilatory prostaglandins such as PGE_2 [7, 8]. However, additional studies suggest that stimulation of prostaglandin production may have inhibitory effects on carrageenin-induced edema [9]. Recent studies in our laboratory have shown that systemic treatment of rats with prostaglandins, especially of the E series, will inhibit vascular permeability changes induced by the vasoactive mediators histamine, serotonin, bradykinin, compound 48/80 and the anaphylatoxin C3a [10]. In this report, we examine the effects of systemic prostaglandin treatment of rats on carrageenin-induced vascular permeability changes.

Carrageenin edema was induced by the intradermal injection of 0.1 ml of 1% carrageenin (viscarin 402) (Marine Colloids, Springfield, NJ), dissolved in phosphate-buffered saline (PBS), pH 7.4, into the hind paw of Sprague–Dawley rats weighing 200–250 g (Charles River Laboratories, Portage, MI). Separate groups of three to five rats were pretreated (subcutaneously) with prostaglandins 1 hr prior to carrageenin injection. Footpad thickness was measured at various times after carrageenin injection with a Mitutoyo micrometer. Prostaglandins (PG) were provided by Dr. John Pike (Upjohn Co., Kalamazoo MI). Student's *t*-test (two tailed) was used to compare PG-treated animals with non-treated control animals.

The effect of systemic treatment of rats with 15-*S*-15-methyl PGE_1 (15-M-PGE_1) on carrageenin-induced footpad edema is shown in Fig. 1. When 15-M-PGE_1 at a dose of 1 mg/kg was administered s.c. 1 hr prior to carrageenin injection, there was significant inhibition of rat footpad edema formation as compared to control values. Both the acute phase (47.1% inhibition at 1 hr, $P < 0.001$) of edema formation mediated by histamine, serotonin, and bradykinin and the later phase (61.0% inhibition at 6 hr, $P < 0.001$) which is inhibited by indomethacin and aspirin were reduced significantly. Inhibition of footpad edema formation by 15-M-PGE_1 was dose dependent with significant inhibition (40.7% at 1 hr, $P < 0.001$ and 37.7% at 6 hr, $P < 0.001$) occurring at a dose of 0.2 og/kg and only