Metabolism of C^{13}-Carboxyl-labeled Malonate by the Intact Mouse* (17936)

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There are a number of papers in the literature which deal with metabolic relationships of malonate other than the inhibition of succinic dehydrogenase. Thus, additional enzymatic inhibitory effects have been suggested(1-3) for this compound. A stimulating effect on the oxygen uptake and carbon dioxide output of sea urchin eggs has been described(4). Malonate has been reported isolated, presumably as a metabolic product, from mold and bacterial cultures(5-8), as well as utilized by such micro-organisms(7,9). According to Vennesland and Evans(10), malonic acid is a product of the metabolism of oxalacetate by a mammalian (pig) heart preparation. In the present investigation the conversion of the carboxyl carbon of malonate to carbon dioxide has been studied in the intact mouse and in dog plasma. The results are interpreted to indicate the probable occurrence in mammalian cells of enzymatic mechanisms for conversion of malonate to carbon dioxide.

Methods. C^{13}-carboxyl-labeled malonic acid containing 7.54 atom % excess C^{13} was synthesized according to the method of Calvin, *et al.*(11). The melting point and acid value of the synthetic material were the same as those of recrystallized Eastman malonic acid. For administration the acid was neutralized with sodium hydroxide and made just colorless to phenolphthalein with 0.1 N HCl.

Experiments on mice. Unfasted mice of the A strain were employed. Malonate (about 1 mM per 100 g of body weight) was injected in a total volume of 1 ml into 3 to 4 subcutaneous sites on the back of the animal. The mouse was placed immediately in a system for collecting the respiratory carbon dioxide in sodium hydroxide. The total carbon dioxide in the alkali was measured by means of the Van Slyke manometric apparatus. C^{13} concentration was determined mass spectrographically. The dose of malonate employed is of the order of ½ to ¼ of the fatal dose for the mice in question. The only effect on the behavior of the animals noted was the production of a degree of lethargy in certain of the mice.

Experiments on dog plasma. Into each of 2 Warburg type flasks of about 170 ml capacity were placed 25 ml of dog plasma plus .25 mM of C^{13}-malonate, and the vessels were shaken at 38°C for 5 hours. At the end of this period, the flask contents were acidified with strong sulfuric acid and the carbon dioxide absorbed into sodium hydroxide for subsequent isotope analysis. The gas phase of one flask was air; of the other, 5% carbon dioxide in oxygen.

Results and discussion. Experiments on mice. In Table I it will be noted that 20-32% of the administered C^{13} was recovered in the respiratory carbon dioxide within 6 hours after injection. Approximately 10% of the administered dose was accounted for in the first hour. The corresponding values decrease with time until, for 2 out of 3 animals, the carbon dioxide in the 6th hour contained

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TABLE I.

Recovery of C13 in Respiratory CO_2 After the Subcutaneous Injection of Approximately* .25 mM of Carboxyl-labeled Malonate Containing 7.54 Atom % Excess C13 in the Whole Molecule.

Mouse	1	2			3			4		
Sex, wt in g	♀, 26	♀, 25			♀, 25			♂, 25		
			C13			C13			C13	
CO_2 collection in hr	Total CO_2 mM	Total CO_2 mM	Atom % excess	% of admin. dose	Total CO_2 mM	Atom % excess	% of admin. dose	Total CO_2 mM	Atom % excess	% of admin. dose
1st	3.67	2.60	.20	9.9	3.93	.15	10.9	3.40	.15	10.
2nd and 3rd	5.41	5.38	.14	14.4	6.53	.06	7.2	5.19	.08	8.2
4th and 5th	5.22	4.09	.08	6.2	5.40	.02	2.	4.81	.02	1.9
6th	2.15	1.70	.06	1.9	2.16	.01	.0	2.12	.01	.0
25th	—	—	—	—	3.28	.00	.0	—	—	—
Total				32.4			20.1			20.1

* Mice 2, 3, and 4 received .23, .24, and .22 mM of malonate, respectively. Mouse 1 received no malonate.

little, if any, labeled carbon. Data for the output of respiratory carbon dioxide of a mouse to which no malonate had been administered are included in Table I to indicate that the carbon dioxide production of the animals receiving malonate was of the same order of magnitude as the normal. It is reasonable to assume that the initial step in malonate metabolism is decarboxylation to acetate. The further reactions of malonate should then be similar to those of acetate, except as the presence of malonate itself has influenced metabolism.†

Experiments on dog plasma. The acid-labile carbon dioxide in each of the 2 flasks contained only .005 atom % excess C^{13}. This value is within the error of the analytical method and indicates little or no decarboxylation of malonate by dog plasma. Given normal plasma bicarbonate levels, decarboxylation of 1% of the added malonate would have been detectable. Hence it appears likely that in the mouse the major site of the conversion of malonate to carbon dioxide is intracellular and that the process is at least in part enzymatic. It is recognized that this inference involves the assumption that the results on dog plasma would hold for not only the extracellular fluids of the mouse, but

† Gould *et al.*‡ have reported that within 4 hours after the intraperitoneal injection of $CH_3C^{14}OOH$ into fasted rats, 87% of the radioactivity was recovered in the respiratory carbon dioxide. In experiments performed after submittal of the present manuscript, recoveries of labeled carbon of the same high order of magnitude were observed in the case of the mice when $CH_3C^{13}OOH$ was administered together with normal malonate. Similar high recoveries might be predicted for any acetate derived metabolically from labeled malonate, if it is assumed, among other things, that the site of malonate decarboxylation is the same as the site of oxidation of exogenous acetate. The finding that only 20-32% of the malonate carboxyl carbon was converted to respiratory carbon dioxide raises the question of the fate of the remaining malonate (urinary excretion, metabolism via pathways not involving acetate, etc.).

‡ Gould, R. G., Sinex, F. M., Rosenberg, I. N., Solomon, A. K., and Hastings, A. B., *J. Biol. Chem.*, 1949, v177, 295.

also the intracellular ones, aside from the enzymatic functions of the latter. Moreover, the degree to which the microorganisms of the gastro-intestinal tract are responsible for the experimental findings has not been evaluated. The parenteral administration of the malonate should reduce the importance of this factor but does not necessarily eliminate it.

Summary. When C^{13} - carboxyl - labeled malonate was injected subcutaneously into mice, 20-32% of the labeled carbon was recovered in the respiratory carbon dioxide in 6 hours.

At body temperature, dog plasma showed little or no ability to decarboxylate malonate.

These results are interpreted to indicate the probable occurrence in mammalian cells of enzymatic mechanisms for conversion of malonate to carbon dioxide.

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Antibiotics in the Treatment of Experimental Acute Hemorrhagic Pancreatitis in Dogs. (17937)

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In a study on the pathogenesis of acute pancreatitis Dragstedt, Haymond, and Ellis (1) reported that devitalized dog pancreas, highly toxic when allowed to autolyze within the peritoneal cavity, was not toxic after being sterilized in the autoclave. Also, sterile fetal pancreas was not toxic when placed within the dogs' peritoneal cavity. Within the uncontaminated pancreas of 13 out of 17 dogs, bacteria common to the intestinal tract were found. *B. welchii* was especially common. No matter what the cause of the necrosis in acute pancreatitis might be, these authors felt that the resulting toxemia was the same and in the major part believed that it was due to the bacterial decomposition products of proteins. These observations suggested to us that antibiotics might be of value in treating experimental pancreatitis in dogs.

Methods. Healthy, adult, dewormed, mongrel dogs were used. Pancreatitis was produced aseptically in the same manner in all the dogs by injecting the animal's own gallbladder bile into its major pancreatic duct and then tying the duct. To produce an hemorrhagic, necrotic pancreatitis with a high fatality rate in non-treated animals it was found necessary to inject a relatively large amount of bile under pressures much higher than have been found normally in the biliary tract of the dog(2). An average of 10.5 cc of bile were injected into the main pancreatic duct extraduodenally through a 20-gauge needle within less than one minute. Pressure readings taken with a mercury manometer attached to a side arm showed pressures of 400 to 500 mm of mercury during the injection. After a satisfactory injection, the pancreatic parenchyma would be stained diffusely; often there would be a mottled brown appearance as described by Mann and Giordano(2).

The animals treated with penicillin were given 300,000 units of penicillin G in 1 cc of oil and wax* intramuscularly, beginning within 4 hours after surgery and continuing once daily for 4 or 5 days. Streptomycin hydrochloride* was given subcutaneously in doses of 0.17 g beginning within 4 hours after the pancreas was injected and continuing every 4 hours, except for the 4:00 A.M. dosage, for

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* Provided by E. R. Squibb and Sons, New York City.