Effect of Metabolites on Accumulation of Citrate in Fluorocitrate-poisoned Rats.* (22461)

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The effect of metabolites of the Krebs cycle on the accumulation of citrate in fluoroacetate-poisoned rats has been reported(1). C_4 metabolites like α-ketoglutarate, succinate, malate and oxalacetate increase the accumulation of citrate in the heart, and to a lesser extent in the kidney, over and above that observed after fluoroacetate alone. The C_2 fragments like acetate and ethanol diminish the accumulation of citrate, possibly by competing with fluoroacetate for coenzyme A, on the assumption that the real poison is fluorocitrate(2). Fluoroacetate can thus be used as a tool in studying the intermediary metabolism of certain substances. For instance, we have found that pyruvate and butyrate behave in the heart like C_4 fragments of the Krebs cycle. The change of pyruvate into oxalacetate may be explained by CO_2-fixation, but the behavior of butyrate cannot be explained on the basis of current concepts of intermediary metabolism. Propanol and propionate behave like C_2 fragments and are the most effective in inhibiting citrate accumulation in fluoroacetate-poisoned rats. The lack of accumulation of citrate in the liver of fluoroacetate-poisoned rats was explained by the assumption that fluoroacetate would be diluted in this chief acetylating organ, in a big pool of C_2 fragments.

If fluoroacetate blocks the Krebs cycle by virtue of a "lethal synthesis" of fluorocitrate (2), an inhibitor of aconitase, it follows that an equimolar dose of fluorocitrate should be more effective than fluoroacetate, since only part of the fluoroacetate would be synthesized into fluorocitrate because of dilution of fluoroacetate in the acetate pool. The results to be reported herein were not as predicted and they show great differences in different organs. An attempt will be made to explain these variations.

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Methods. Adult male albino rats, 4 to 7 months old, were used. Although these were of a different strain than those used in the previous study(1) yet the "resting" tissue citrate values were the same in both strains. Synthetic fluorocitric acid(3), 12.7 mg per kg, was neutralized and injected intraperitoneally 30 minutes after the subcutaneous injection of the metabolite. This quantity of fluorocitrate is double the molar dose of fluoroacetate used previously since Peters(4) has shown that synthetic fluorocitrate is half as effective as the "enzymatic" fluorocitrate in inhibiting the aconitase of kidney particles. The rest of the experimental technic has been described previously(1).

Results. In 6 control rats without poison the average citrate content of the tissues in μg per gram was as follows: Heart, 23 ± 2.5; Kidney, 45 ± 5.5; Liver, 46 ± 3.7; Brain, 41 ± 1.8; Plasma, 30 ± 2. The results with fluorocitrate are summarized in Table I.

Heart. Fluorocitrate increases the heart citrate much less than fluoroacetate, 150 μg compared to ca. 1000 μg. Part of the 150 μg of citrate are due to plasma and extracellular fluid in the heart but this does not account for the whole increase since the plasma citrate content of these rats is only 276 ± 20 μg per ml. It appears then that the heart is very slightly permeable to fluorocitrate. The increase in citrate content after butyrate is not surprising since unpoisoned hearts react likewise(1). Starvation increases the citrate level but this is observed also in nonpoisoned animals, (unpublished experiments).

Brain. There is little accumulation of citrate in the brain of fluorocitrate-poisoned rats. The small increase may be accounted for by the plasma and extracellular fluid content of the brain. However, the magnitude of these figures is unknown to us. Furthermore

TABLE I. Citrate Accumulation in Tissues of Fluorocitrate-Treated Rats after Injection of Various Metabolites. Mean values ± S.E. are given in μg/g wet tissue. Unless otherwise stated, 2.25 millimoles of metabolite were inj./100 g body wt.

Metabolite	No. of animals	Heart	Kidney	Liver	Brain
Saline controls	27	148 ± 13	1760 ± 56	1790 ± 19	77 ± 4
Succinate	10	71 ± 8	731 ± 55	1040 ± 106	68 ± 3
Malate	9	147 ± 14	1470 ± 74	1095 ± 74	117 ± 16
Acetate*	10	187 ± 18	1780 ± 140	1317 ± 86	105 ± 10
Ethanol†	10	134 ± 17	1797 ± 81	527 ± 85	66 ± 4
Propanol	10	98 ± 15	1638 ± 66	1058 ± 81	84 ± 7
Propionate	3	132 ± 21	2027 ± 129	1194 ± 69	96 ± 20
Acetone†	10	166 ± 20	1830 ± 87	1507 ± 56	79 ± 6
Butyrate†	5	497 ± 16	2720 ± 195	1370 ± 156	74 ± 8
Starvation	10	213 ± 12	1665 ± 40	316 ± 42	80 ± 7

* 3.1 millimoles/100 g body wt.
† 4.5 *Idem*

Peters *et al.*(4) obtained values of ca. 250 μg per gram after using 4 times the dose employed here. It would seem then, that the brain is also only slightly permeable to fluorocitrate.

Kidney. There is more accumulation of citrate in the kidney with fluorocitrate than with fluoroacetate. Here again, the increase in citrate after butyrate reminds one of the condition obtained in the nonpoisoned kidney. The C_4 fragments cause a decrease in accumulated citrate.

Liver. Here, high values, ca. 1800 μg, are obtained with fluorocitrate, whereas with fluoroacetate there is a slight or no increase, (4,5). The effect of metabolites on the liver citrate were not predictable according to our experience with the heart and kidney of fluoroacetate-poisoned animals, for here both the C_4 and C_2 fragments of the Krebs cycle inhibited the citrate accumulation in the liver, ethanol being most active. A possible explanation for these findings will be given below. Starvation for 48 hours has a profound influence on citrate accumulation reducing it to one-sixth the value obtained in fed animals.

Discussion. The fact that there is no significant accumulation of citrate in the heart of fluorocitrate-poisoned rats is most probably attributable to the lack of diffusibility of fluorocitrate and does not suggest that fluoroacetate and fluorocitrate act by two different mechanisms. In unpublished work from this laboratory on the effect of fluoroacetate on the dog heart-lung preparation it was shown that the heart citrate can increase 20-fold without a trace of it diffusing into the serum. The same considerations may explain the insignificant rise in brain citrate after fluorocitrate.

C_4 intermediates of the Krebs cycle cause a decrease of citrate accumulation in the liver and kidney of fluorocitrate-poisoned animals instead of an increase as with fluoroacetate. We venture the following explanation for the difference. Owing to the poor diffusibility of fluorocitrate the more diffusible C_4 fragments, injected 30 minutes before fluorocitrate, may have increased the citrate content to such an extent as to protect aconitase against slowly diffusible fluorocitrate. The action of the C_2 fragments was also not predictable. We did not expect a decrease of accumulated citrate as in fluoroacetate poisoning, since here there is no competition with fluoroacetate for coenzyme A. However, a decrease did take place. Experiments are in progress to elucidate this "anomalous" behavior of C_2 fragments in fluorocitrate poisoning as well as that of butyrate in fluoroacetate poisoning.

Starvation reduces citrate accumulation in fluorocitrate-poisoned rats to one-sixth the value found in fed rats. This may suggest that in starvation, the Krebs cycle operates at a reduced rate.

Summary. 1) Fluorocitrate causes much less accumulation of citrate in the heart than does fluoroacetate. This is most likely due to relative impermeability of the heart to fluorocitrate. The same appears to apply to the brain. 2) C_2 as well as C_4 fragments of the Krebs cycle decrease the accumulation of citrate in the liver of fluorocitrate-poisoned ani-

mals. 3) Forty-eight hours' starvation reduces the citrate accumulation in the liver of fluorocitrate-poisoned rats to one-sixth the value obtained in fed animals.

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Effects of X-Irradiation on Androgenic Response of Seminal Vesicles of the Castrate Rat.* (22462)

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In investigating cytological effects of testosterone propionate on secretory epithelium of rat seminal vesicles, Cavazos and Melampy (1) observed no mitoses in epithelium of castrates or 24- and 36-hour hormone-treated animals. However, mitotic activity of 7.5% was noted 60 hours after initial hormone treatment. Following this maximum there was a sharp decline in mitoses and in animals treated with male hormone for 20 days a value of 0.1% was observed. Colchicine was used in these experiments.

Regressed seminal vesicles of the castrate rat provide useful test tissue for such investigation because cells of secretory epithelium are in the resting stage and can be reactivated with androgen. Observations are reported on effects of various levels of X-irradiation on 60-hour response of seminal vesicles to testosterone propionate (T.P.).† Data were obtained on mitotic index and cell height of secretory epithelium as well as total nitrogen content of the organ.

Materials and methods. 159 rats of Holtzman strain were used and fed Purina Laboratory Chow. At time of castration average animal weights ranged from 266 to 312 g and seminal vesicles were allowed to regress 20 days before X-irradiation and/or hormone treatment. The initial dose of androgen was administered at time of irradiation. Hormone-treated castrates received daily 500 μg of T.P. in oil injected subcutaneously, total amount 1500 μg. Control groups received 0.05 ml of oil daily and no irradiation. Experimental animals were sacrificed 60 hours following treatment. Animals were exposed to X-rays from General Electric Maxitron operated at 130 kvp and 10 ma with 0.25 mm Al filtration and at 20 cm target distance. Nembutal anesthesia was employed during irradiation (2.5 mg/100 g body wt). Animals were shielded with lead except in pelvic area. Individuals used to furnish data on mitotic index and cell height of secretory epithelium were injected subcutaneously with 0.1 mg of colchicine per 100 g body weight 6 hours prior to killing according to Burkhart(2). Percentages of mitotically dividing nuclei, mitotic index, and cell heights were determined by procedure described earlier(1). Total nitrogen data were obtained by a micro-Kjeldahl method.

Results. The results of this investigation are summarized in Tables I and II. Data are presented as means with their standard errors.

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† Peranderen, Ciba Pharmaceutical Products, Summit, N. J.