Inhibition of oxidative mechanism by 3-fluoropyruvate*

Fluoropyruvate has been found to produce convulsions in rats without the concomitant rise in citric acid such as is observed in fluoroacetate poisoning, thereby indicating that in the body it did not undergo oxidative decarboxylation represented by the equation:

$$FCH_2COCOOH + H_2O \rightarrow FCH_2COOH + CO_2 + 2\,H^+ + 2e$$

The mechanism of generating the fluoroacetyl fragment thus remained inoperative. However, fluoropyruvate inhibited the aerobic oxidation of pyruvate *in vitro* and of ^{14}C-labeled acetate *in vivo*[1,2].

Using the pigeon breast muscle pyruvic oxidase preparation of SCHWEET[3], as modified by SANADI[4], we have now shown that fluoropyruvate inhibits the anaerobic oxidation of pyruvate when indophenol is used as electron acceptor (Table I).

TABLE I

INHIBITION OF PYRUVIC OXIDASE BY 3-FLUOROPYRUVATE

System	*mμmoles Dye reduced/2 min*	*% Inhibition*
Enzyme alone	1.26	—
Enzyme + 0.3 μmoles fluoropyruvate	1.26	—
Enzyme + 1.0 μmoles fluoropyruvate	2.10	—
Enzyme + 1.65 μmoles pyruvate	11.25	—
Enzyme + 1.65 μmoles pyruvate + 0.3 μmoles fluoropyruvate	6.42	48%
Enzyme + 1.65 μmoles pyruvate + 1.0 μmoles fluoropyruvate	3.84	83%

The reaction mixture consisted of potassium phosphate buffer, pH 7.5 (4 μmoles) $MgCl_2$ (6.6 μmoles), cocarboxylase (66 μg), 2–6 dichlorophenolindophenol to give an initial optical density of approximately 0.600, and pyruvic oxidase (450 μg of dry weight of protein), in 1 ml final volume. Amount of dye reduced was calculated from the decrement in optical density for the 2-minute period following the addition of the enzyme. Measurements were carried out on a Beckman DU Spectrophotometer at 600 mμ.

Further evidence of inhibition of aerobic pyruvate oxidation has been obtained from experiments with liver mitochondria prepared in sucrose or mannitol according to the method modified after LEUTHARDT AND MULLER[5]. Fluoropyruvate (1 μmole) inhibited pyruvate (9 μmoles) in both tris and phosphate buffers to 45–75%. The oxidation of fumarate (20 μmoles) was inhibited to 55–80% and 35–50% by fluoropyruvate, 3 and 1 μmoles, respectively. Fluoropyruvate also inhibited the entry of fluoroacetate into the tricarboxylic acid cycle. This was judged both from its effect upon citrate accumulation in presence of fluoroacetate (Table II) and also by enzymic estimation of the fluorocitrate formed. For instance, with kidney particles, using malate as substrate in presence of fluoroacetate (49 μmoles) and fluoropyruvate (8.3 μmoles), there was a reduction of approximately 60% in the amount of fluorocitrate formed, as judged by its effect on brain particles. Contrary to the *in vivo* results[6], the rat liver mitochondria of the fasted male rats appear to activate fluoroacetate, although results with neither rat[1] nor pigeon liver homogenate[7,8,9] would indicate the formation of fluoroacetylhydroxamate.

In keeping with this difference between acetate and fluoroacetate, Table II shows that acetate has slightly increased the effect of fumarate on the mitochondrial respiration. However, it does not seem to be available for the cycle in presence of fluoropyruvate (3 μmoles) even at a high acetate concentration (30 μmoles). Consequently, the inhibition is not released. At a lower inhibitory level of fluoropyruvate (1 μmole), the addition of acetate (10 μmole) significantly reversed the effect of the inhibitor. In other experiments, fluoropyruvate also effected an extensive dephosphorylation of ATP in an inorganic-phosphate free mitochondrial system in presence of fumarate and pyruvate.

* This work was supported by a grant, B-608, from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S. Public Health Services.

TABLE II

INHIBITION OF THE ENTRY OF FLUOROACETATE AND ACETATE IN THE TCA CYCLE BY 3-FLUOROPYRUVATE

	Substrate	*Inhibitors*	*(μmole)*	*Citrate (μmole)*	Q_{O_2}	% *Inhibition*
A.						
	Fumarate	—	—	0.65	70.7	—
	Fumarate	Fluoroacetate	3.3	2.22	63.8	9.8
	Fumarate	Fluoropyruvate	1.0	0.27	45.7	35.4
	Fumarate	Fluoroacetate + fluoropyruvate	3.3 1.0	0.531	34.5	51.9
B.						
	Fumarate	—	—	—	101	—
	Fumarate	Fluoropyruvate	1.0	—	62.4	38.1
	Fumarate	Fluoropyruvate	3.0	—	22.8	77.4
	Fumarate + acetate (10 μmoles)	—	—	—	125.0	—
	Fumarate + acetate (10 μmoles)	Fluoropyruvate	1.0	—	109.2	12.6
	Fumarate + acetate (30 μmoles)	—	—	—	123.4	—
	Fumarate + acetate (30 μmoles)	Fluoropyruvate	3.0	—	36.9	70.1

The vessels contained: tris-(hydroxymethyl)-amino methane buffer (0.02 *M*) at pH 7.28; $MgCl_2$ 6 μmoles; KCl 120 μmoles; potassium fumarate 20.0 μmoles; Na_2ATP 0.5 μmoles. Sodium acetate, fluoroacetate and fluoropyruvate as indicated. The inhibitors were added in the flask. All solutions were made up in tris-buffer. To the flasks, 1.0 ml of mitochondrial suspension from the liver of overnight-fasted male Wistar rats was added. In experiments under A, mannitol (840 μmoles per flask) was present; under B, sucrose (744 μmoles per flask) was present. Warburg's direct manometric technique was used at 37° C. The Q_{O_2} values are expressed as μl O_2/h/mg mitochondrial nitrogen. The citrate determinations were carried out by a method modified after BUFFA AND PETERS[6].

Our results, therefore, would indicate that 3-fluoropyruvate not only inhibits the pyruvic oxidase system, but also has an inhibiting effect at sites controlling the entry of acetate and fluoroacetate into the oxidative cycle.

EMERY M. GAL
ALAN S. FAIRHURST
ROBERT E. SMITH

Departments of Pharmacology and Physiology, Medical Center, University of California, Los Angeles, Calif. (U.S.A.)

and

RUDOLPH A. PETERS

Biochemistry Department, A.R.C. Institute of Animal Physiology, Cambridge (England)

1 E. M. GAL, R. A. PETERS AND R. W. WAKELIN, *Proc. Biochem. Soc.*, 58 (1954) xiii; *Biochem. J.*, (in press) 1956.
2 R. A. PETERS, *Bull. Johns Hopkins Hosp.*, 97 (1955) 21.
3 V. JAGANNATHAN AND R. S. SCHWEET, *J. Biol. Chem.*, 196 (1952) 551.
4 J. W. LITTLEFIELD AND D. R. SANADI, *J. Biol. Chem.*, 199 (1952) 65.
5 F. LEUTHARDT AND A. F. MULLER, *Experientia*, 4 (1948) 478.
6 P. BUFFA AND R. A. PETERS, *J. Physiol.*, 110 (1949) 488.
7 R. A. PETERS, *Brit. Med. J.*, 2 (1952) 1165.
8 J. F. MORRISON AND R. A. PETERS, *Biochem. J.*, 58 (1954) 473.
9 R. O. BRADY, *J. Biol. Chem.*, 217 (1955) 213.

Received March 6th, 1956