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# Studies with Specific Enzyme Inhibitors

## XIII. Kinetics of Nonenzymatic Decarboxylation of Fluorooxalacetic Acid

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SUMMARY

The kinetics of spontaneous and metal ion-catalyzed decarboxylation of oxalacetic and fluorooxalacetic acids was determined by enzymatic analyses of keto acids. Decarboxylation of fluorooxalacetate to enol-fluoropyruvate and subsequent tautomerization to keto-fluoropyruvate were followed by spectrophotometric analysis of enol-fluoropyruvate and some of its metal ion complexes. Rate equations for the specific case of two consecutive irreversible reactions, where $k_1 > k_2$, were derived and tested experimentally. Nonenzymatic catalytic decarboxylation of fluorooxalacetate accounts for fluoropyruvate formation in the presence of liver parenchymal cell suspensions.

### INTRODUCTION

Substitution of one hydrogen atom by fluorine converts oxalacetic acid into a highly effective competitive inhibitor of malate dehydrogenase (EC 1.1.1.37) with a $K_i$ value on the order of 0.1 $\mu$M (1, 2). Because of its powerful inhibitory effect on malate dehydrogenase, fluorooxalacetic acid appeared to be suitable as an enzyme probe in metabolic systems in which the rate-limiting role of this enzyme was to be explored (see ref. 3). Prior to the application of FOAA[2] in cellular systems, it was of importance to ascertain both its specificity and metabolic stability in various enzyme systems. The NADP$^+$-dependent decarboxylating malate dehydrogenase (EC 1.1.1.40) was inhibited only by concentrations of FOAA several orders of magnitude higher than necessary to block malate dehydrogenase activity (4). Fluorooxalacetate also condenses with acetyl-CoA enzymatically to yield a noninhibitory isomer of fluorocitrate (5), but this reaction is slow and may account only for a small portion (10–15%) of FOAA disappearance in relatively short (30–40-min) experiments. A more efficient "detoxification" pathway of FOAA is its transamination with glutamate to yield unstable fluoroaspartate, which instantly decomposes to OAA + $NH_3$ + HF (6). This reaction may be estimated to take place at about 20% of the rate of transamination between OAA and glutamate, but is severely limited in tissues by endogenous glutamate levels. Simultaneous application of FOAA and difluorooxalacetate, an inhibitor of glutamate-oxalacetate aminotransferase (EC 1.1.1.40) with a $K_i$ of about 1 $\mu$M (7), appeared to be a predictably

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[2] The abbreviations used are: FOAA, monofluorooxalacetic acid; OAA, oxalacetic acid.

effective method for prolonging the biological stability of FOAA in cellular systems. Further experiments revealed the instability of FOAA toward nonenzymatic decarboxylation, a reaction presumably analogous to the well-known decarboxylation of OAA (8). In order to compare the stability of OAA and FOAA, the kinetics of decarboxylation of FOAA to fluoropyruvate had to be studied in some detail. Apart from the relevance of these results to the stability of FOAA in biological systems, the kinetic analysis of decarboxylation of FOAA to enol-fluoropyruvate and subsequent tautomerization to keto-fluoropyruvate may be applicable to any two consecutive irreversible first-order systems in which detection of the intermediate is analytically feasible, and therefore may have some general usefulness.

## MATERIALS AND METHODS

*Purification of FOAA*. The preparation of FOAA by the hydrolysis of its diethyl ester (1, 9) was improved by transesterification in trifluoracetic acid, a procedure which eliminates the use of HCl and the problem of its removal.

Diethyl fluorooxalacetate was dissolved in trifluoroacetic acid and allowed to stand overnight at room temperature. Most of the ethyl trifluoroacetate (b.p. 62°) and trifluoracetic acid (b.p. 74°) was subsequently removed by distillation. The FOAA, which crystallizes after this step, contained about 10% oxalic acid and 20% fluoropyruvic acid as detected by paper electrophoresis (5).

Further purification of FOAA was carried out as follows: 75 g of FOAA were dissolved in refluxing ether (300 ml). The first crop of crystals, which were formed by chilling the ether solution, was composed of 85% oxalic acid. The addition of chloroform (100 ml) gave a second crop, and the further addition of pentane (100 ml) to the filtrate, a third crop of crystals. The combined second and third crops of crystals (50 g) consisted of 85% FOAA, 15% fluoropyruvic acid, and only traces of oxalic acid (less than 1%). A second recrystallization from ether with chloroform yielded FOAA of 95% purity with 3-5% fluoropyruvic acid as contaminant, and no oxalic acid.

$$C_4H_3FO_5 \cdot 2H_2O$$

Calculated: C 25.82, H 3.79, F 10.21

Found: C 25.68, H 4.30, F 10.36

Traces of fluoropyruvate may be formed by spontaneous decarboxylation of FOAA during prolonged storage, and it is practically impossible to avoid this trace contamination. For enzymatic studies, its removal from a freshly prepared solution of FOAA is best accomplished by enzymatic reduction of fluoropyruvate to fluorolactate by NADH plus lactate dehydrogenase (EC 1.1.1.28), followed by removal of the enzyme protein by passing the solution through a small column of Sephadex G-25 (10-cm length, 1-cm width, equilibrated with $H_2O$) at 0–4°. Pyridine nucleotides may be removed by adsorption on activated charcoal. Traces of fluorolactate, which is a relatively weak inhibitor of lactate dehydrogenase (10), do not interfere in most instances when the inhibitory effect of FOAA on malate dehydrogenase is being studied.

*Enzymatic assay for FOAA and fluoropyruvate*. Since FOAA and fluoropyruvate are substrates of malate dehydrogenase and lactate dehydrogenase, respectively (see ref. 3), their quantitative determination is readily accomplished by enzymatic optical tests, just as for their nonfluorinated homologues. Samples (10 μl) of reaction mixtures containing the keto acids were transferred to cuvettes charged with 0.17 mM NADH in 1 ml of Tris-HCl (0.1 M, pH 7.4) or EDTA (0.1 M, pH 7.4) buffer. The oxidation of NADH by FOAA was measured after the addition of 2 μl of beef heart malate dehydrogenase[3] (Boehringer-Mannheim), and NADH oxidation by fluoropyruvate was assayed after the addition of 2 μl of lactate dehydrogenase[3] (Boehringer-Mannheim). The reaction for FOAA and fluoropyruvate was complete in about 10 min (at 27°). Changes in absorbance at 340 nm were measured in Zeiss PMQ II spectrophotometers (1-cm light path), and the concentrations of keto acids were calculated in the usual manner.

[3] Undiluted commercial preparations of dehydrogenases are suitable for assays.

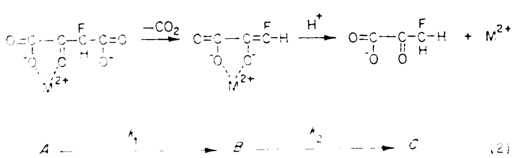
*Determination of first-order rate constants.* Both the decarboxylation of FOAA to fluoropyruvate and the enol-keto conversion of the latter followed first-order kinetics, as determined by direct enzymatic analyses for fluoroketo acids or by recording of absorbance changes characteristic for enol-fluoropyruvate (see RESULTS). Five (or, in a few cases, four) enzymatic analyses were performed in the course of two half-time periods, and the results were fitted to the equation

$$A = A_0e^{-kt} \quad (1)$$

where $A_0$ is the initial concentration of FOAA (or OAA, if decarboxylation of OAA was measured), $k$ is the first-order rate constant, and $A$ is the FOAA (or OAA) concentration at any time $t$. $A_0$ and $k$ were obtained by a least-squares approximation program,[4] with the aid of a PDP-12 digital computer. Data from kinetic experiments were acceptable only if the standard error of the estimates of $A$ were within 5%.

The rates of absorbance changes characteristic for the decarboxylation of FOAA and subsequent enol-keto tautomerization of fluoropyruvate were measured in a Unicam SP-800-B spectrophotometer or, if assayed at a fixed wavelength, in a Gilford recording spectrophotometer.

Analyses of results were performed according to a model of two consecutive first-order processes. The chemical reactions involved are illustrated in Eq. 2.



(2)

where $k_1$ and $k_2$ are first-order rate constants: $k_1$ is the rate constant for the formation of $B$, and $k_2$ is the rate constant of the decay of $B$, where $B$ denotes enol-fluoropyruvate. The solution of these two consecutive first-order processes can be derived from the equation which expresses the rate of formation of $B$ (11) as follows.

$$B = A_0\frac{k_1}{k_2 - k_1}(e^{-k_1t} - e^{-k_2t}) \quad (3)$$

Set

$$r = \frac{k_1}{k_1 - k_2}$$

Then

$$B + rA = A_0re^{-k_2t}$$

or

$$\ln(B + rA) = \ln(A_0r) - k_2t$$

as

$$k_1 > k_2$$

Equation 3 can be written

$$B = A_0\frac{k_1}{k_1 - k_2}(e^{-k_2t} - e^{k_1t})$$

and

$$B = rA_0(e^{-k_2t} - e^{k_1t})$$

where $r > 0$. $B' \equiv rA_0e^{-k_2t}$, which is the "long-time" asymptotic limit of $B$. Then $B' - B = rA_0e^{-k_2t}$ and $\ln(B' - B) = -k_1t + \ln rA_0$, which determines $k_1$ just as $\ln B' = -k_2t + \ln rA_0$ determines $k_2$. It should be noted that the molar absorbance ($\epsilon_M$) of $B$ can be calculated from this last expression (see also later). In a plot of $\ln B'$ in $t$ (Fig. 2), the intercept at $t = 0$ is $\ln B_0'$, where $B_0' \equiv \epsilon_BrA_0$; therefore a knowledge of $r$ [$= k_1/(k_1 - k_2)$] and the molarity of $A_0$ determines the $\epsilon_M$ of $B$, which is $\epsilon_B = B_0'/rA_0$.

The time ($t_{max}$) required to reach the maximal concentration of $B$ (i.e., $B_{max}$) is related to $k_1$ and $k_2$ by the following equation.

$$t_{max} = \left(\frac{1}{k_2 - k_1}\right)\ln\frac{k_2}{k_1} = \frac{1}{k_2}(r - 1)\ln\left(\frac{r}{r - 1}\right) \quad (4)$$

Values of $t_{max}$ were calculated on a PDP-12 digital computer. A graphical method of obtaining these parameters is illustrated in Fig. 2. It is apparent[5] that the maximal

[4] We are indebted to Dr. H. M. Martinez for the program.

[5] We are indebted to Dr. L. Peller for calling our attention to this relationship.

concentration of $B$, i.e., $B_{max}$ (which is the concentration of $B$ at $t_{max}$ when $dB/dt = 0$), is a function of the rate constants and of $t_{max}$ and can be expressed as follows:

$$\ln \frac{A_0}{B_{max}} = k_2 t_{max}$$

By substituting Eq. 4 for $t_{max}$ in this expression, we obtain Eq. 5.

$$\frac{B_{max}}{A_0} = \left(\frac{k_2}{k_1}\right)^{k_2/(k_1-k_2)} = \left(\frac{r-1}{r}\right)^{r-1} \quad (5)$$

In Eq. 5, $A_0$ is known (in molar concentrations), the rate constants are known, and $B_{max}$ is read in absorbance units at the peak of experimentally obtained curves (as shown in Fig. 3). Therefore, the concentration of $B$ at $t_{max}$ (i.e., $B_{max}$) can be calculated. With both the concentration and absorbance of $B_{max}$ known, its molar absorption coefficient ($\epsilon_M$) can be calculated from the Beer-Lambert equation. Calculated $\epsilon_M$ values are shown in Table 1.

## RESULTS

*Decarboxylation of OAA and FOAA without added metal ions.* In these experiments (Fig. 1), the first-order rate constants of decarboxylation of OAA or FOAA were determined by direct enzymatic analyses of keto acids. For OAA, the maximal rate was found between pH 3.1 and 3.6. The pH maximum of decarboxylation of FOAA was at 2.6, which is about the midpoint of the previously reported pK values (2.2 and 3.3; see ref. 1) of this fluoroketo acid.

The stability of 0.01 M FOAA was also determined at 25° and pH 7.15 in three different buffers: 0.1 M Tris-HCl, sodium phosphate, and sodium EDTA. The half-life of FOAA at 25° in all three buffers was the same (300–320 min). A study of the influence of temperature on the stability of FOAA at pH 7.15 gave the following results. The half-life of FOAA at 40° was 30 min; at 25°, 5.3 hr; and at 0°, 7 days. At −20°, no decarboxylation could be detected after 2 weeks.

*Effect of $Mn^{2+}$ on decarboxylation of OAA and FOAA.* The maximal rate of decarboxylation of OAA in the presence of 5 mM $MnCl_2$ occurred at pH 4.6. The rate of

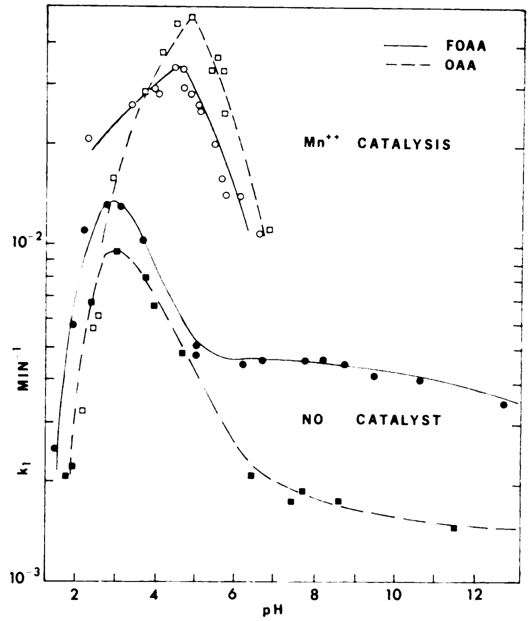



FIG. 1. *Rates of decarboxylation of FOAA and OAA as a function of pH*

The reaction mixture (10 ml) contained 10 mM FOAA (or OAA) and 0.1 M buffer, maintained at 30°. When the effect of $Mn^{2+}$ was to be measured, it was added as $MnCl_2$ in a final concentration of 5 mM. Rate constants were determined by direct enzymatic assays for FOAA and OAA (see MATERIALS AND METHODS). Ordinate: first-order rate constants of decarboxylation (on a $\log_{10}$ scale). Abscissa: pH. The buffers used were chloracetate (pH 2–3), formate (pH 3–4), acetate (pH 4–5), maleate (pH 5–6), Tris-HCl (pH 7–9), and bicarbonate-carbonate (pH 9–12).

reaction in the presence of $Mn^{2+}$ was about 3.2 times faster than without any added divalent ion. Similarly, the decarboxylation of FOAA was enhanced by a factor of 2.6 in the presence of 5 mM $MnCl_2$. The pH maximum of the $Mn^{2+}$-catalyzed decarboxylation was 3.4.

It should be noted that $Mn^{2+}$ is unstable above pH 6.0; thus its catalytic effect cannot be studied reliably in the more alkaline pH range. Trivalent manganic ion formed above pH 6.0 by aerobic oxidation is apparently stabilized by carboxylic acids, producing yellow complexes (12).

The effects of pH on the spontaneous and $Mn^{2+}$-catalyzed decarboxylation ($k_1$) of

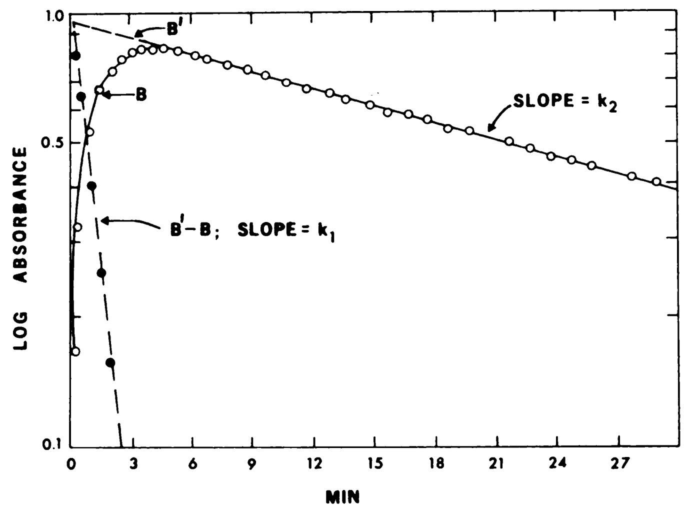



FIG. 2. *Graphical method for calculation of first-order rate constants of decarboxylation ($k_1$) of FOAA to enol-fluoropyruvate and subsequent tautomerization ($k_2$) of the product*

Derivations are given in MATERIALS AND METHODS. The reaction mixture (3 ml) contained 0.33 mM FOAA and 1 mM aluminum sulfate in 10 mM sodium formate (pH 2.6), maintained at 40°. Absorbance changes were recorded at 240 nm. Ordinate: log absorbance at 240 nm. Abscissa: time in minutes.

OAA and FOAA determined with the aid of enzymatic analyses for keto acids are illustrated in Fig. 1.

*Effect of amines on decarboxylation of OAA and FOAA.* It is well known that the decarboxylation of OAA is greatly accelerated by various amines. A 5.4-fold increase in the rate of decarboxylation of OAA was reported in the presence of aniline (13). On the other hand, no significant increase in the spontaneous decarboxylation of FOAA was effected by aniline, cyclohexylamine, or imidazole (0.1 M amine, 0.01 M fluorooxalacetate, 0.1 M sodium acetate buffer, pH 5.0, at 30°).

*Decarboxylation of FOAA and subsequent enol-keto conversion of fluoropyruvate as determined by spectrophotometry.* Since FOAA in neutral solutions contains no appreciable enol (14), it was possible to follow by direct spectrophotometry the rate of its decarboxylation ($k_1$) to enol-fluoropyruvate and to determine the subsequent rate of conversion of the enol-fluoropyruvate to the keto tautomer ($k_2$). The two processes followed a consecutive first-order rate pattern (see MATERIALS AND METHODS). Both the spontaneous and metal-catalyzed reactions were studied. When the logarithm of absorbance change at 225 nm (absorption maximum of the enol of fluoropyruvate) was plotted against time, a characteristic curve was obtained. The first, rapidly ascending part indicated the rate of decarboxylation of FOAA, while the descending part was a measure of the rate of disappearance of the enol peak of fluoropyruvate (see Fig. 2). From the rate of formation and decay of fluoropyruvate (see Eq. 2), both $k_1$ and $k_2$ were calculated. A direct recording of absorbance changes (recorded with the Unicam spectrophotometer) of a solution of FOAA in the presence of 1 mM $CuSO_4$ (at 225 nm) is illustrated in Fig. 3. The peak of the curve corresponds to the light absorption of $B_{max}$. The results shown in Fig. 2 have been calculated from the ascending and linear parts of the descending portion of experimental values as shown in Fig. 3. The shape of the curve depends on the presence of various catalytically active ions, which influence both the decarboxylation of FOAA and, to a lesser extent, the enol-keto conversion of fluoropyruvate. Results expressing absorption maxima ($\lambda_{max}$ of $B$), first-order rate con-

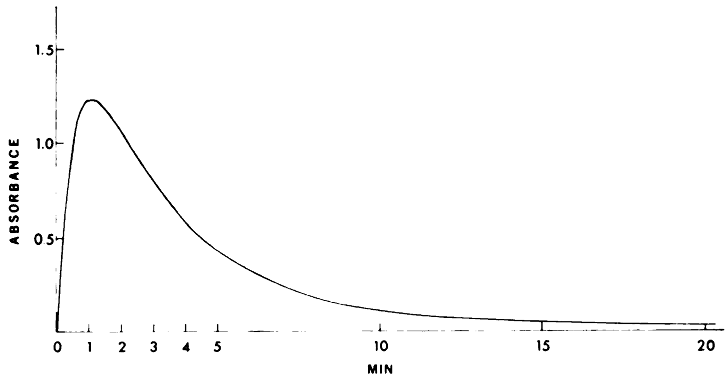



FIG. 3. *Direct spectrophotometric assay of decarboxylation of FOAA and tautomerization of fluoropyruvate*

The rate of formation and decay of the $Cu^{2+}$ complex of enol-fluoropyruvate is indicated by the time function of absorbance changes at 225 nm. The reaction mixture (3 ml) contained 0.33 mM FOAA and 1 mM $CuSO_4$ in 10 mM sodium trifluoroacetate buffer (pH 2.6) at 40°. Ordinate: absorbance at 225 nm. Abscissa: time in minutes.

TABLE 1

*Formation and decay of the enol of fluoropyruvate from FOAA*

Kinetic parameters of decarboxylation of FOAA and tautomerization of enol-fluoropyruvate. For calculation of the rate constants, $t_{max}$, and $\epsilon_{max}$, see MATERIALS AND METHODS. The reaction mixture (3 ml) contained 0.33 mM FOAA and 1 mM metal ion (acetate or chloride) in 10 mM trifluoracetate buffer (pH 2.6), maintained at 40°. The concentration of FOAA was raised to 1 mM for the $Fe^{3+}$-catalyzed reaction in order to diminish the interference caused by absorbance of $FeCl_3$ at 510 nm.

| Catalyst | $\lambda_{max}$ | $k_1 \times 10^2$ | $k_2 \times 10^2$ | $t_{max}$ observed | $t_{max}$ calculated | $\epsilon_{max}$ calculated |
|---|---|---|---|---|---|---|
| | *nm* | *min*$^{-1}$ | *min*$^{-1}$ | *min* | *min* | $mM^{-1}\ cm^{-1}$ |
| None | 225 | 6.73 | 1.83 | 26.8 | 26.6 | 5.1 |
| $Cu^{2+}$ | 225 | 31.00 | 27.30 | 1.1 | 1.1 | 1.3 |
| $Zn^{2+}$ | 225 | 12.00 | 2.89 | 15.0 | 15.7 | 3.5 |
| $Mn^{2+}$ | 225 | 7.92 | 1.78 | 25.0 | 24.3 | 5.0 |
| $Mg^{2+}$ | 225 | 7.62 | 1.65 | 25.0 | 25.6 | 5.2 |
| $Al^{3+}$ | 240 | 88.30 | 3.00 | 3.8 | 4.0 | 4.0 |
| $Fe^{3+}$ | 510 | 17.30 | 3.00 | 12.5 | 12.3 | 1.0 |

stants, $t_{max}$, and calculated $\epsilon_M$ values of $B_{max}$ are shown in Table 1. With the exception of $Al^{3+}$ and $Fe^{3+}$, all absorption maxima were at 225 nm. The formation of a purple $Fe^{3+}$ complex with FOAA has been observed previously (see ref. 1). The identity of the enol complex of fluoropyruvate was established by direct enzymatic assays for fluoropyruvate. Although the enol peak of fluoropyruvate decayed with time, a reaction characterized by $k_2$, no net disappearance of fluoropyruvate could be detected by the lactate dehydrogenase assay (see MATERIALS AND METHODS), clearly indicating that fluoropyruvate did not undergo subsequent decomposition in the course of spontaneous or any of the metal ion-catalyzed decarboxylations of FOAA. Both enzymatic methods of keto acid analyses and direct spectrophotometry yielded identical rate constants.

*Stability of FOAA and fluoropyruvate in suspensions of rat liver cells.* The stability of both FOAA and fluoropyruvate (prepared by decarboxylation by heating FOAA in aqueous solution at 100° for 15 min) was determined by enzymatic analyses of these fluoroketo acids (see MATERIALS AND METHODS) following incubation with a suspension of isolated rat liver parenchymal cells in balanced salt solution, as described by Berry and Friend (15), at 37°. The over-all metabolic activity of liver cell suspensions was measured in air in Gilson respirometers. In the absence of added substrate, the rate of $O_2$ uptake (expressed as micromoles of $O_2$ per minute per gram of liver cells) was 1.86, and the addition of 1.25–5 mM FOAA had no significant effect (rates varied between 1.68 and 1.91) during 40 min of incubation. In contrast to FOAA, oxalacetate (5 mM) stimulated $O_2$ uptake by 30–40% under comparable conditions, while fluoropyruvate (5 mM) depressed $O_2$ uptake by 26%. The spontaneous rate of decarboxylation of FOAA was the same with or without liver cells during 40 min of aerobic incubation at 37°, corresponding to the rate calculated for the catalytic decarboxylation of FOAA determined in the absence of liver cells. The decarboxylation of FOAA in the presence of liver cells is characterized by the same temperature-dependent half-life as determined for solutions of FOAA (see first section under RESULTS). On the other hand, added fluoropyruvate was quantitatively recovered after 40 min of incubation with liver cells. About 25% of the FOAA which disappeared during incubation could not be accounted for as fluoropyruvate at the end of 40 min in the presence of liver cells. Suspensions of liver cells contain endogenous substrates to provide glutamate sufficient to initiate the known enzymatic conversion of FOAA to fluoroaspartate, followed by decomposition to $NH_3$ plus oxalacetate (see ref. 6). It is probable that the transaminative route of FOAA degradation, together with the enzymatic reduction of FOAA to fluoromalate, can account for the major part of the metabolic disposition of FOAA by liver cells. There was no evidence to indicate enzymatic decarboxylation of FOAA.

## DISCUSSION

Our results indicate that the mechanism of decarboxylation of FOAA follows the pattern previously established for OAA (8, 16). The kinetics of two consecutive first-order processes which characterize the conversion of FOAA to keto-fluoropyruvate was tested by independent analytical procedures which permitted the calculation of the molar concentration of the intermediate product. This kinetic approach may yield useful results for the estimation of the intermediate in any similar irreversible two-step process. Analysis of the effect of pH on the rate of spontaneous and metal ion-catalyzed decarboxylations showed that in the former the reactive species was the monoanion, while in the presence of metal ions it was the dianion (see Fig. 1). These conclusions confirm previous reports dealing with the decarboxylation of OAA (8, 17, 18). It is likely that in the absence of an electron-attracting metal ion at characteristic (for monoanion) pH values, the protonated carboxyl group adjacent to the carbonyl exerts a weak electron attraction, promoting labilization of the carboxyl group adjacent to the fluorine-substituted carbon atom of FOAA. On the other hand, acceleration of the decarboxylation of FOAA or OAA by metal ions requires the dianion, and the reactive species is the metal chelate of the keto tautomer. The rate of decarboxylation shows characteristic differences, depending on the nature of the metal ion. We have chosen excess concentrations of metal ions in order to provide a basis for comparison of various metal ions. Calculation of the molar absorbance of the intermediate, i.e., the metal complex of enol-fluoropyruvate, yielded—as expected—similar values (see Table 1), with the exception of $Cu^{2+}$ and $Fe^{3+}$. Since $k_1$ and $k_2$ in the case of $Cu^{2+}$ catalysis are nearly the same, it is predictable that calculations derived for conditions under which $k_1 > k_2$ should not be valid. $Fe^{3+}$ absorbs light in the same region as its enol-fluoropyruvate complex, and this interference prevents determination of the absorbance of the $Fe^{3+}$–enol-fluoropyruvate complex itself. These reasons explain the anomalous experimental values obtained for $Cu^{2+}$ and $Fe^{3+}$. Comparison of the rates of metal ion-catalyzed decarboxylation of OAA and FOAA shows that reaction rates are related to the presence of the keto tautomer of both oxalacetates. According to Fig. 1, rate constants for decarboxylation above pH 6.0 are about twice as large for FOAA as for oxalacetate. Since the concentration of the enol of oxalacetate shows pH dependence above pH 6.0 (18), which is opposite to the pH dependence of the rate of its decarboxylation (19), it is apparent that the decreased rate of decarboxylation of oxalacetate is related to the lower concentration of the keto tautomer. In calculating rate constants, no correction was made for enolization, which, in the case of OAA above pH 7.0, may approach 50%, but is negligible for FOAA (14). Since the usual spectrophotometric method of calculating the concentration of the enol tautomer of OAA was shown to be unreliable, and accurate determination by deuterium exchange under all conditions is difficult (see ref. 14), rate constants for the decarboxylation for OAA were calculated in terms of the total dianion.

It is of interest that amines (e.g., aniline) rapidly decarboxylate OAA, but do not accelerate this process in case of FOAA. This difference provides a method of selective removal of OAA if present together with FOAA.

As a consequence of the present studies, the application of FOAA as an enzyme inhibitor in tissue systems may be evaluated more accurately. There seems to be no enzyme system in liver cells that accelerates the spontaneous decarboxylation of FOAA; furthermore, fluoropyruvate is stable in liver cell suspensions and is not converted to fluorolactate or metabolized to fluoroacetyl-CoA. These properties of fluoropyruvate suggest its usefulness as an enzyme inhibitor in isolated liver cells. On the other hand, interpretation of the metabolic effects of FOAA has to take into account its decarboxylation to fluoropyruvate, which can be calculated from the results presented in this paper.

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