THE SPECIFICITY AND KINETICS OF ACONITASE

by

H. KACSER

The Queen's University, Belfast (Northern Ireland)

It is now generally accepted that the enzyme aconitase catalyses the equilibrium between citric, *cis*-aconitic and D-*iso*citric acids. Attempts to show that two enzymes are involved (*cf.* JACOBSOHN[1,2]) have not been substantiated. It has been pointed out (OGSTON[3]) that the stereochemical results of the reaction can be explained by assuming that the *cis*-aconitate molecule is adsorbed by "threepoint attachment" on the enzyme. According to the orientation of approach of water molecules either citric or D-*iso*citric acid is produced.

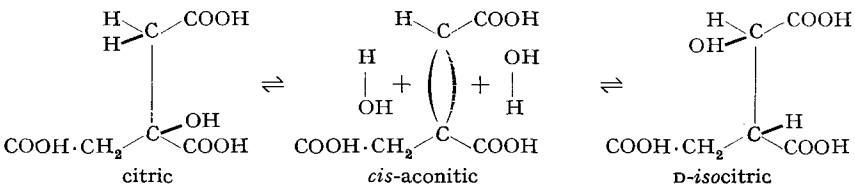

Fig. 1

If the enzyme surface is represented by the plane of the paper, approaches by the water molecules can only proceed from "above" and the steric result will only depend on the orientation of addition to the double bond. The question of whether the water molecule reacts from the aqueous phase or is first adsorbed, however, remains unanswered. In the former case the rate of addition (though not necessarily the equilibrium concentrations) for the two isomers should be equal, while in the latter case the asymmetry of the enzyme surface makes this highly improbable. There is evidence (MARTIUS AND LEONHARDT[4]) that the initial rate is the same for both reactions.

It is desirable to substantiate this stereochemical reasoning by a kinetic analysis of the reaction scheme and compare it with the available data.

The equilibrium values for the three acids are given (KREBS AND EGGLESTON[5]) as follows: D-*iso*citric acid 6.2%, *cis*-aconitic acid 4.3%, citric acid 89.5% ± 10%. In view of the more recent determination[6] of 6.6% for D-*iso*citric acid and the mean deviation for citric acid, the following values for the equilibrium concentrations will be used: D-*iso*citric acid 6.6%, *cis*-aconitic acid 4.3%, citric acid 89.1%.

The course of the reaction, starting with *cis*-aconitate, has been followed[4] but only the graphs are available. The lack of recorded data precludes the proper estimation of the initial rate, but the appearance of a maximum in the concentration-time curve can equally well be used in the subsequent analysis. The position of this maximum is estimated at 10 hours.

References p. 415.

These four values are sufficient to calculate the course of the reaction.

In a recent publication FRIEDRICH-FRESKA AND MARTIUS[7] have offered a solution of the kinetics in a manner somewhat similar to the one developed here (see also MARTIUS AND LYNEN[8]). Their analytic procedure differs, however, in several important points affecting the quantitative values of constants in the rate equations. As a consequence they reach conclusions which are opposed to those which follow from our analysis. A discussion of their paper is, for convenience, deferred until the present treatment has been presented.

There is no evidence that the reaction takes place to any measurable extent in the absence of the enzyme. We may therefore assume that reaction takes place only among the species adsorbed on the enzyme surface, although the fraction of such adsorbed molecules is small compared with the fraction in solution. We therefore have the system:

$$\begin{array}{ccccc} A & & C & & I \\ a_I \downarrow\uparrow d_I & & a_A \downarrow\uparrow d_A & & d_C \downarrow\uparrow a_C \\ I_S & \underset{k_I'}{\overset{k'}{\rightleftharpoons}} & A_S & \underset{k_C'}{\overset{k'}{\rightleftharpoons}} & C_S \end{array} \tag{1}$$

The letters a and d stand for the adsorption and desorption constants respectively and the letters k for the rate constants for the reaction on the surface. I, A and C refer to *iso*citric, aconitic and citric acid respectively and the subscript s indicates the adsorbed species.

If the total available enzyme surface is taken as unity and the fractions covered by each species at any instant are I_S, A_S and C_S, then

$$\text{Fraction of free surface} = 1 - I_S - A_S - C_S \tag{2}$$

Taking the case of *iso*citric acid, we can formulate the rate of change of the adsorbed species in the following way: the rate of adsorption is proportional to the free fraction and to the concentration of the species in solution

$$\text{Rate of adsorption} = a_I \left[1 - (I_S + A_S + C_S)\right] I \tag{3}$$

The rate of desorption and the rate of reaction are proportional to the fraction covered by the species

$$\text{Rate of desorption} = d_I I_S \tag{4}$$

$$\text{Rate of reaction} = k_I' I_S \tag{5}$$

Since adsorbed acid is also formed by reaction from aconitic acid, we have

$$\text{Rate of formation} = k' A_S \tag{6}$$

Therefore the total rate of change is

$$\frac{\mathrm{d}\, I_S}{\mathrm{d}\, t} = a_I \left[1 - (I_S + A_S + C_S)\right] I - d_I I_S - k_I' I_S + k' A_S \tag{7}$$

The reaction rate constants will in general be much smaller than those for the adsorption and desorption process since much higher activation energies are encountered in chemical changes than in surface attachments. Since I_S and A_S are also very small, the last two terms make a very small contribution to the total rate and as they are of opposite sign we may neglect them.

References p. 415.

$$\frac{\mathrm{d}\,I_S}{\mathrm{d}\,t} \approx a_I\left[1 - (I_S + A_S + C_S)\right] I - d_I I_S \tag{8}$$

This means that the rate of change of surface concentration is nearly entirely determined by the sorption process. With the high substrate concentrations used the equilibrium is attained very rapidly compared with any chemical change. For sorption equilibrium we equate the rate to zero and obtain:

$$I_S = \frac{a_I\left[1 - (I_S + A_S + C_S)\right] I}{d_I} \tag{9}$$

Although the relative proportions of I_S, A_S and C_S will change during the course of the reaction, their sum will remain substantially constant and under, the experimental conditions, will be high.

Equation (9) may therefore be expressed as

$$I_S = k^* I \tag{10}$$

In other words the fraction of the adsorbed species is proportional to the concentration in solution. The same arguments can be shown to apply to aconitic and citric acids. We may therefore use the concentrations in solution instead of the surface fractions for the kinetic expressions of the reaction rates.

It is convenient to sum up the assumptions underlying the subsequent treatment.

1. One enzyme site only is concerned with the reaction of all three species.

2. Their rates are proportional to their concentration in solution.

3. There is an equal probability of the alternative water orientations for the addition to the double bond in *cis*-aconitate. (Equal rate constants for the changes $A \rightarrow I$ and $A \rightarrow C$)

4. The dehydrations of citric and *iso*citric acid are governed by separate and different rate constants.

With these assumptions and the equilibrium values given above we may formulate the rate equations for the system:

$$I \underset{k_I}{\overset{k}{\rightleftharpoons}} A \underset{k_C}{\overset{k}{\rightleftharpoons}} C \tag{11}$$

If we start with A (*cis*-aconitic) at unit concentration at $t = 0$, then at time t' we have

$$x_I \underset{k_I}{\overset{k}{\rightleftharpoons}} 1 - (x_C + x_I) \underset{k_C}{\overset{k}{\rightleftharpoons}} x_C \tag{12}$$

where x_C and x_I are the concentrations of citric and *iso*citiric acids (expressed in mole. fractions) respectively. The rate equations for the formation of the two isomers are then:

$$\frac{\mathrm{d}\,x_C}{\mathrm{d}\,t} = k\left[1 - (x_C + x_I)\right] - k_C\, x_C \tag{13}$$

$$\frac{\mathrm{d}\,x_I}{\mathrm{d}\,t} = k\left[1 - (x_C + x_I)\right] - k_I\, x_I \tag{14}$$

References p. 415.

The following further relations hold:

$$K_C = k/k_C = \frac{x_C^\infty}{1 - (x_C^\infty + x_I^\infty)} \tag{15}$$

$$K_I = k/k_I = \frac{x_I^\infty}{1 - (x_C^\infty + x_I^\infty)} \tag{16}$$

where K is the equilibrium constant and x^∞ the equilibrium concentrations of the two species.

The two differential equations (13) and (14) can be brought into the following forms by suitable multiplications and subtractions.

$$(k + k_I)\frac{\mathrm{d}\,x_C}{\mathrm{d}\,t} - \frac{k\,\mathrm{d}\,x_I}{\mathrm{d}\,t} + x_C(kk_C + kk_I + k_Ck_I) = kk_I \tag{17}$$

$$-k\frac{\mathrm{d}\,x_C}{\mathrm{d}\,t} + (k + k_C)\frac{\mathrm{d}\,x_I}{\mathrm{d}\,t} + x_I(kk_C + kk_I + k_Ck_I) = kk_C \tag{18}$$

These are now of the form

$$L\frac{\mathrm{d}\,x_C}{\mathrm{d}\,t} + M\frac{\mathrm{d}\,x_I}{\mathrm{d}\,t} + Rx_C = E$$

$$M\frac{\mathrm{d}\,x_C}{\mathrm{d}\,t} + N\frac{\mathrm{d}\,x_I}{\mathrm{d}\,t} + Sx_I = F$$

and are soluble (LAMB[9]) with solutions:

$$x_C = E/R + A_1\,\mathrm{e}^{\lambda_1 t} + A_2\mathrm{e}^{\lambda_2 t}$$

$$x_I = F/S + B_1\,\mathrm{e}^{\lambda_1 t} + B_2\mathrm{e}^{\lambda_2 t}$$

where λ_1 and λ_2 are the solutions of the quadratic

$$\lambda^2(LN - M^2) + \lambda(LS + NR) + RS = 0$$

and A_1, A_2, B_1 and B_2 are obtained by substituting λ_1 and λ_2 respectively in

$$(L\lambda + R)A + M\lambda B = 0$$

Substitution of the appropriate constants and making use of relation (15) and (16) gives

$$x_C = \frac{K_C}{K_C + K_I + 1} + A_1\mathrm{e}^{\lambda_1 t} + A_2\mathrm{e}^{\lambda_2 t} \tag{19}$$

$$x_I = \frac{K_I}{K_C + K_I + 1} + B_1\mathrm{e}^{\lambda_1 t} + B_2\mathrm{e}^{\lambda_2 t} \tag{20}$$

and

$$\lambda_{1,2} = \frac{-k(2K_CK_I + K_C + K_I) \pm k\sqrt{(K_I - K_C)^2 + (2K_CK_I)^2}}{2K_CK_I} \tag{21}$$

K_C and K_I are known from the concentrations of the final products and k may be evaluated from the initial rate or, as will be shown later, from the position of the maximum in $\frac{\mathrm{d}\,x_I}{\mathrm{d}\,t}$. Using the values $x_C^\infty = 0.891$, $x_I^\infty = 0.066$, $x_A^\infty = 0.043$, we obtain

References p. 415.

$K_C = 20.72$ and $K_I = 1.53$ and therefore from (21) $\lambda_1 = -2.39\,k$ and $\lambda_2 = -0.31\,k$. Evaluating A_1 and A_2 we obtain $A_1 = 0.742\,B_1$ and $A_2 = -0.142\,B_2$, so that (19) and (20) become

$$x_C = 0.891 + 0.742\,B_1\,e^{-2.39\,kt} - 1.42\,B_2\,e^{-0.31\,kt} \quad (22)$$

$$x_I = 0.066 + B_1\,e^{-2.39\,kt} + B_2\,e^{-0.31\,kt} \quad (23)$$

From the condition $t = 0$, then $x_C = 0$ and $x_I = 0$, we can eliminate B_1 and B_2 and obtain the equations

$$x_C = 0.891 - 0.339\,e^{-2.39\,kt} - 0.552\,e^{-0.31\,kt} \quad (24)$$

$$x_I = 0.066 - 0.456\,e^{-2.39\,kt} + 0.390\,e^{-0.31\,kt} \quad (25)$$

If data for the initial rate were available, such a value could be substituted for k. A more reliable value can be calculated from the estimated value of the time at which the maximum occurs. The condition for the maximum is

$$\frac{d\,x_I}{d\,t} = 0$$

When equation (25) is differentiated this gives

$$k\,t_{max} = 1.06 \quad (26)$$

The numerical value for t_{max} from the curves given by MARTIUS AND LEONHARDT is 10 hours and therefore

$$k = 0.106 \text{ hours}^{-1} \quad (27)$$

The equations for citric and *iso*citric acid respectively are therefore

$$x_C = 0.891 - 0.339\,e^{-0.255\,t} - 0.552\,e^{-0.033\,t} \quad (28)$$

$$x_I = 0.066 - 0.456\,e^{-0.255\,t} + 0.390\,e^{-0.033\,t} \quad (29)$$

The course of the two equations is given in Fig. 2 together with the experimental points[4] as far as this was possible. The agreement is fair but there are obvious deviations which require discussion.

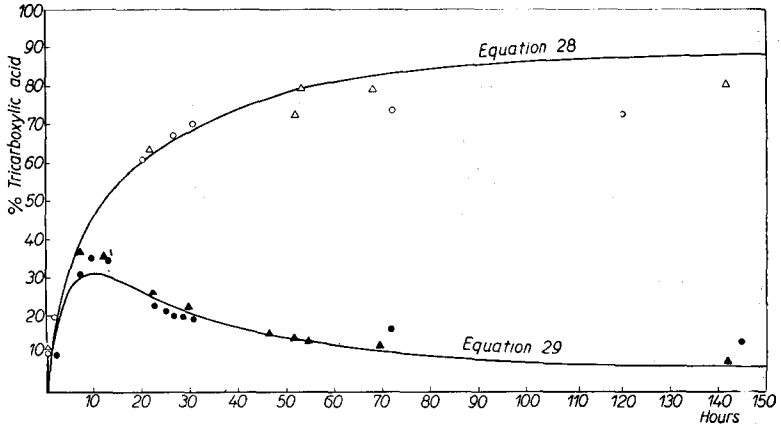

Fig. 2. △ and ▲ Enzyme from bean extract ○ and ● Enzyme from liver extract (MARTIUS AND LEONHARDT[4])

References p. 415.

There are several factors which make the experimental points unreliable, some affecting both isomers others concerned with *iso*citric acid only.

It is clear that in the two enzyme preparations investigated the citric acid does not approach the equilibrium value of $\approx 89\%$. As the authors admit, this may be due to the conversion of some *cis*-aconitate into the *trans* form prior to the experiment. Since the *trans* acid is not acted upon by the enzyme, the system would start with less than 100% of reacting material. This would require the "scaling up" of all values proportionately if the percentage *trans* acid were known.

The presence of *trans*-aconitic acid has, however, a further effect. It has been shown (SAFFRON AND PRADO[10]) that the presence of *trans* acid inhibits the rate competitively and also appears to result in the approach to a new lower equilibrium value for citric acid. There is no evidence what the effect on the *iso*citric acid concentration is.

The presence of *trans* acid is therefore sufficient to account for the deviation of the experimental points for citric acid. Additional factors influence the recorded points for *iso*citric acid.

The extreme sensitivity of the rotation of the *iso*citrate-molybdate complex to external conditions makes a direct translation of rotational data to concentrations very difficult.

It must be assumed that MARTIUS AND LEONHARDT used the value of $[\alpha]_D = -670°$ for the specific rotation in arriving at the concentration values for *iso*citric acid recorded in their diagram. The evidence (KREBS AND EGGLESTON[5,6]) shows, however, that the specific rotation varies with the concentration of *iso*citrate and with the concentration of citrate if it is present. Since both these quantities change absolutely and relatively to one another throughout the experiment, the use of a single value of $[\alpha]$ for the calculations of concentration is not admissible. Equations (28) and (29) on the other hand depend only on the *position* of the maximum and not on the particular concentration value assigned to it. This position of the rotational maximum is but slightly influenced by the effect of concentration changes on the rotation, especially since the *iso*citrate concentration varies insignificantly at that point.

The calculations based on this value and on the equilibrium data, therefore, appear to be more reliable than the experimental points. The equilibrium concentrations are known with a higher degree of accuracy, especially, since the value of $x_I^\infty = 6.6\%$ (EGGLESTON AND KREBS[6]) has been calculated by taking the known concentration conditions into account.

A much more detailed study of the rotational changes with external conditions would be required before the equations could be subjected to a rigorous comparison. In view of the above considerations and the general uncertainty of the experimental values, the agreement is sufficiently good.

It must be pointed out that the value $k = 0.106$ hours^{-1} is not an absolute rate constant but only holds for the particular enzyme concentration used in the experiments. Since there are no data available for pure aconitase, k cannot be converted to unit enzyme concentration. Similarly the values $k_C = 0.005$ hours^{-1} and $k_I = 0.069$ hours^{-1} which may be derived from relations (15) and (16) are relative only. Equations (28) and (29) are therefore particular cases for the (unknown) concentrations of these workers.

On the other hand, equations (24) and (25) are independent of a particular value for k, having been derived by using the equilibrium concentrations only. Similarly,

References p. 415.

although the *position* of the maximum will vary with enzyme concentration, the *value* of the maximum *iso*citrate concentration is independent of this and is:

$$\underset{\text{(max.)}}{\text{D-}iso\text{-citrate}} = 31\% \tag{30}$$

This value agrees well with recent available data (FRIEDRICH-FRESKA AND MARTIUS[7], $\approx$ 29%; MARTIUS AND LYNEN[8], $\approx$ 31%) obtained under different conditions from those on which these calculations are based.

The ratio

$$k_I/k_C = 13.6 \tag{31}$$

which derives from relations (15) and (16) is also substantially independent of enzyme concentration at high substrate concentration. For purified enzyme preparations RACKER[11] reports a ratio $k_I/k_C = 7$–7.5. But since DL-*iso*citrate was used, only half the *iso*citrate concentration was available for reaction. If therefore k_I is doubled this gives $k_I/k_C = 14$–15 in good agreement with our value.

It may therefore be stated that equations (24) and (25) give the kinetic expressions for the two isomers although more accurate data may slightly alter the absolute value of the constants. They represent the general case until the molecular weight of pure aconitase has been determined.

This analysis has important corollaries concerning the mechanism of the catalysis.

It confirms the conclusion that one enzyme only is involved in the change.

The assumption of the treatment was that both acids were formed with the same rate constant k. The agreement implies that the addition of water to the double bond must take place from the aqueous phase and not by prior adsorption on the enzyme. The asymmetry of the enzyme surface makes it extremely unlikely that the possible adsorption site is *equally* suitable for either orientation of the water molecule.

Any poisoning can only affect the single adsorption site of the molecules and should therefore influence citric and *iso*citric rates in the same way*.

The comparatively slight changes in the equilibrium proportions with temperature (JACOBSOHN[2]) find an explanation. Changes in temperature will affect the absolute magnitude of the adsorbed amount (depending on the free energy of adsorption) and the number of collisions with the requisite activation energy. Both these factors will influence the rate at which the equilibrium is approached. But temperature changes will not affect the equal probability of the alternative orientations of approach by the water molecules. Changes in composition can therefore only arise if the two rate constants for the reverse step, k_C and k_I, change differently with temperature. Over the small temperature range available for these studies this is unlikely to have a significant effect.

The specificity of aconitase is not "kinetic" by the preferential catalysis of one particular reaction path since the proportions of citric and *iso*citric acids are only determined by the free energy difference of the two species. Since $\Delta F_{CI} = \Delta F_C - \Delta F_I$, where $\Delta F = -RT \ln K$, the ratio of the equilibrium constants is given by:

$$K_C/K_I = e^{-\Delta F_{CI}/RT} \tag{32}$$

From this it follows that $\Delta F_{CI} = -1570$ cal/mol (33)

* In a publication (LOTSPEICH, PETERS AND WILSON, *Biochem. J.*, 51 (1952) 20) which has appeared since this paper was submitted, it has in fact been shown that the four processes, a. C → I, b. I → C, c. A → C, and d. A → I are all inhibited by a fluorotricarboxylic acid ("inhibitor fraction") isolated from tissue poisoned with fluoroacetate. Members of each pair, a and b, c and d respectively are inhibited to the same degree, although the latter pair is less sensitive to the inhibitor than the former.

References p. 415.

As far as these two reactions are concerned, aconitase acts as a symmetrical catalyst, increasing the rate of both equally. There are unfortunately no rate-temperature data available so that it is not possible to say in what way the enzyme affects the activation energy.

The specificity is, however, "stereo-chemical" insofar as it adsorbs the *cis*-aconitate molecule on one "side" only and thereby prevents the production of any of the other isomers. The asymmetry of the surface site insures that the alternative mode of adsorption, the "reverse side" of the aconitate molecule, is not possible. Such mode of adsorption would produce citric and L-*iso*citric acids, while water addition without adsorption would produce citric acid and an optically inactive mixture of the four *iso*-acid isomers.

The treatment of the kinetics which FRIEDRICH-FRESKA AND MARTIUS[7] develop results in an equation of the same form as our equation (25) although the numerical values of the constants are considerably different. Their derivation differs from ours in being based on somewhat questionable assumptions concerning the identity of the MICHAELIS constants and in the use of older equilibrium values ($x_I^\infty = 7.7\%$; $x_C^\infty = 89.2\%$; $x_A^\infty = 3.1\%$)[4]. There also seems to be a misprint or mistake in numerical evaluation of their equation (9) since it gives a value of 20% *iso*citric acid instead of zero for $t = 0$. Their equations are not in good agreement with their own data which fact they claim as evidence against the correctness of system (11).

Another objection raised by these authors against system (11) is that the calculated curve for the change citrate → *iso*citrate shows a small induction period while the experimental curve "proceeds apparently without such"[7] (see also MARTIUS AND LYNEN[8], p. 194). This objection can, however, not be seriously maintained in view of the relatively large scatter recorded for their initial points and the early and very slight inflexion of the theoretical curve. The difficulties in assessing rapidly changing *iso*citrate concentrations from the rotation of the molybdate complex, discussed above, rule out the possibility of attaching significance to these small and random deviations.

The conversion of citric into *iso*citric acid also provides the authors with their main argument for the assumption that a "direct conversion" of the two acids, not via aconitate, takes place. Although the general slope of the calculated curve agrees with the experimental points, the absolute value of the initial rate appears to be much greater than that expected from the rate of aconitate → *iso*citrate and the assumptions of system (11). As in a previous paper, no details of calculations are given and it must be assumed that the *single* value $[\alpha]_D = -670°$ was used in converting rotations into concentrations and hence in arriving at the value of the initial rate. The objections to using a single value have already been pointed out but it is possible to show in what direction the error, so introduced, will tend. It is known[5,6] that the effects on the *iso*-citrate-molybdate complex are as follows:

a. The specific rotation increases with citric acid concentration at constant *iso*-citrate concentration.

b. The specific rotation decreases with *iso*citrate concentration at constant citrate concentration.

Since in the initial stages of the citrate → *iso*citrate reaction the citrate concentration is greater and the *iso*-citrate concentration smaller than the equilibrium values, *both* these factors will produce a specific rotation greater than that under equilibrium conditions. Calculations based on the latter value will therefore give apparent concen-

References p. 415.

trations higher than the true values. The high initial rate which FRIEDRICH-FRESKA AND MARTIUS report is doubtless due to this error in the calculation of the concentration. Their observation can therefore not be taken as evidence for the incorrectness of the underlying assumptions of the kinetic analysis.

In any case, the lack of correlation between experimental points and their derived equations causes the authors to abandon the reaction scheme represented by the system (11). Although we have shown that the analysis based on this system does yield equations which are in satisfactory agreement with experiment, it is useful to comment on the alternative scheme suggested by these workers, particularly since they use this as an argument for a certain reaction sequence *in vivo*.

They propose the system:

$$\begin{array}{ccc} & A_S & \\ \swarrow & & \searrow \\ I_S & \rightleftharpoons & C_S \end{array} \qquad (33$$

and suggest[7] that a "direct transformation" from citric to *iso*citric acid takes place *via* an unspecified intermediate product. It is difficult to imagine the stereochemical nature of this intermediate since the process involves the physical transposition of an OH group for an H radicle from one C-atom to another. Such transposition is, however, possible by the removal of the elements of water from the two adjacent C-atoms and their replacement in opposite orientation with respect to the molecule. The intermediate compound so produced is, however, nothing more than the adsorbed aconitate molecule with which our analysis has been concerned. It is irrelevant to the kinetic expressions whether such adsorbed aconitate is produced by dehydration on the surface or by adsorption from solution. The rate of reaction is only determined by the concentration on the surface of each species independent of their past history or origin.

The second and more serious difficulty arises when it is attempted to apply the kinetics of system (33) quantitatively. The authors state[7] that it is not possible to obtain an even approximate agreement between observed equilibrium values and initial rates on the assumption of identical MICHAELIS constants. In order to obtain near agreement they estimate that the surface concentrations are in the ratio $A_S:I_S:C_S = 1:1:2$ and choose, quite arbitrarily, $A^\infty = 5\%$, $I^\infty = 7\%$ and $C^\infty = 88\%$. Such a procedure is particularly objectionable in this case where slight changes can have a profound effect on the rather sensitive exponential functions. But even with this device they admit to a somewhat rough agreement with experiment.

As a result of their acceptance of system (33) the authors conclude that aconitic acid is a bye-product of the citric $\rightleftharpoons$ *iso*citric acid change when the reaction takes place under physiological conditions. The conclusions concerning the primary formation of citric acid as against aconitic acid in the tricarboxylic acid cycle is more fully stated by MARTIUS AND LYNEN[8] but the evidence is largely based on the paper discussed here. In view of the present analysis and criticism such conclusions do not appear to be sound. It must further be borne in mind that no argument based on the isolated aconitase system can be applied simply to conditions *in vivo* where steady state rather than equilibrium conditions apply. There is contradictory evidence (KREBS[12], KREBS AND EGGLESTON[13], STERN AND OCHOA[14]) as to the primary product in the tricarboxylic acid cycle but the experimental facts are more firmly in favour of citric acid. This leads to the following scheme for the reaction sequence:

References p. 415.

→ citrate
↓
cis-aconitate
↓
D-*iso*citrate
↓

Aconitic acid is therefore seen to be a necessary intermediate product in the tricarboxylic acid cycle.

SUMMARY

The kinetic equations for the system citrate ⇌ *cis*-aconitate ⇌ *iso*citrate, catalysed by aconitase, are developed on the assumption of a single adsorption site and the identity of the rate constants for the aconitate → citrate and the aconitate → *iso*citrate reactions. The available kinetic data and stereochemical results are in agreement with this formulation. It is concluded that water addition takes place, not by prior adsorption on the enzyme, but from the aqueous phase and that *cis*-aconitate is a necessary intermediate product in the tricarboxylic acid cycle.

RÉSUMÉ

Les équations kinétiques du système citrate ⇌ *cis*-aconitate ⇌ *iso*-citrate, catalysé par l'aconitase, sont établis sur l'hypothèse d'un lieu d'adsorption unique et de l'identité des constantes de vitesse pour les reactions aconitate → citrate et aconitate → *iso*-citrate. Les équations s'accordent avec les données kinétiques et stéreochimiques. On conclut que l'addition de l'eau n'a pas lieu par l'adsorption sur l'enzyme mais de la solution et que *cis*-aconitate est un produit intermédiaire nécessaire dans le cycle des acides tricarboxyles.

ZUSAMMENFASSUNG

Die kinetischen Gleichungen für das durch Aconitase katalysierte system Citrat ⇌ *Cis*-Aconitat ⇌ *Iso*-citrat werden aufgestellt. Sie sind bedingt durch die Annahmen dass eine einzelne Adsorptionsstelle gebraucht wird und dass die Geschwindigkeitkonstanten für die Reaktionen Aconitat → Citrat und Aconitat → *Iso*-citrat gleich sind. Die Gleichungen stimmen mit den kinetischen und stereochemischen Versuchsresultaten überein. Es folgert dass die Wasseraufnahme nicht über an Ferment gebundenes Wasser, sondern von der Lösung stattfindet. *Cis*-Aconitat erscheint als ein notwendiges Zwischenprodukt des Tricarbonsäure Cyclus.

REFERENCES

1 JACOBSOHN, *Bull. soc. chim. biol.*, 42 (1940) 48.
2 JACOBSOHN, *Enzymologia*, 8 (1940) 327.
3 OGSTON, *Nature*, 167 (1951) 693.
4 MARTIUS AND LEONHARDT, *Z. physiol. Chem.*, 278 (1943) 208.
5 KREBS AND EGGLESTON, *Biochem. J.*, 37 (1943) 334.
6 EGGLESTON AND KREBS, *Biochem. J.*, 45 (1949) 578.
7 FRIEDRICH-FRESKA AND MARTIUS, *Z. Naturforschung*, 6b (1951) 296.
8 MARTIUS AND LYNEN, *Advances in Enzymol.*, 10 (1950) 167.
9 LAMB, *Infinitesimal Calculus*, 3rd. Ed. (1919) 447.
10 SAFFRON AND PRADO, *J. Biol. Chem.*, 180 (1949) 1301.
11 RACKER, *Biochim. Biophys. Acta*, 4 (1950) 211.
12 KREBS, *The Harvey Lectures*, 44 (1948–'49) 165.
13 KREBS AND EGGLESTON, *Biochem. J.*, 42 (1948) 294.
14 STERN AND OCHOA, *J. Biol. Chem.*, 191 (1951) 161.

Received January 7th, 1952