STUDIES ON THE LIPOLYTIC ENZYME ACTION

IV. EXAMPLE OF MATHEMATICAL TREATMENT OF AN ENZYMATIC PROCESS CONSISTING OF CONSECUTIVE REACTIONS

by

F. SCHØNHEYDER, K. OLESEN AND K. VOLQVARTZ
Biochemical Institute and Physical Institute, Aarhus University (Denmark)

In Paper III[1] of this series it was shown that by the action of liver esterase tripropionyl glycerol is hydrolyzed via 1,2-dipropionyl and 2-monopropionyl glycerol. Other intermediate degradation products need not be considered. The rate of hydrolysis of these esters showed a considerable difference, the 2-monopropionyl glycerol being split extremely slowly by an amount of liver esterase having a great capacity for splitting tripropionyl glycerol; the 1,2-compound has a position between these two.

The enzymatic hydrolysis of tripropionyl glycerol by liver esterase is an example of a process in which enter consecutive reactions, proceeding at very different rates. HALDANE[2] points out the difficulties that are connected with the treatment of the kinetics of such a type of process giving rise to differential equations which are not in general integrable.

In the case of the triglycerides such reactions have not been studied closely, and there is need of further work in this field both theoretical and practical.

The object of the present work has been to study the kinetics of the hydrolysis process of the separate esters in the degradation of tripropionyl glycerol in the hope that it would be possible on the basis of the results from the experiments with the separate esters to put forward a reaction scheme leading to an equation which fits the reaction course for the hydrolysis of tripropionyl glycerol much further than the liberation of one acid equivalent.

METHODS

Liver esterase was obtained by extracting chopped, acetone and ether dried rabbit liver tissue with 1% NaCl solution.

Tripropionyl, 1,2-dipropionyl and 2-monopropionyl glycerol were synthesized as described previously[1].

The enzymatic hydrolysis was followed at pH 7.10 and 22° by means of the *continuous titration technique*[3].

EXPERIMENTAL AND RESULTS

Kinetics of the reaction: Tripropionyl glycerol $\rightharpoonup$ 1,2-dipropionyl glycerol + propionic acid (tri $\rightharpoonup$ di)

In Paper II[4] of this series experiments were described in which the hydrolysis course

References p. 91.

of rac. 1-monocaprylyl glycerol (enzyme: liver esterase) might be rendered by the equation

$$Et = A \ln \frac{a}{a-x} + Bx \text{ or } t = A' \ln \frac{a}{a-x} + B'x \quad (1)$$

where

$$A' = \frac{1}{Ek_1}\left(1 + \frac{k_{-1}}{k_2}a\right) \quad (2)$$

and

$$B' = \frac{1}{Ek_2}\left(1 - \frac{k_{-1}}{k_1}\right) \quad (3)$$

a = initial substrate concentration. x = concentration of acid liberated at time t.

Equation (1) is obtained by assuming the following two-step reaction mechanism:

$$OH^- + \text{Ester} + X_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_2 + C^- \quad (\pm 1)$$

$$X_2 \xrightarrow{k_2} X_1 + \text{gl} \quad (+2)$$

X_1 and X_2 are different forms of the enzyme. C^- = fatty acid, gl = glycerol. OH^- concentration constant during the experiment.

By differentiation of (1), putting $x = 0$, we get

$$E\frac{1}{v_0} = \frac{1}{k_2} + \frac{1}{a}\frac{1}{k_1} \quad (4)$$

i.e. $1/v_0$ (v_0 = initial velocity) varies linearly with $1/a$. When $1/v_0$ is plotted as ordinate against $1/a$ as abscissa, the abscissa intercept = k_1/k_2.

In Table I are shown the data from a typical experiment with tri. The values of

TABLE I

HYDROLYSIS OF TRIPROPIONYL GLYCEROL (1.838 mmol per litre) IN THE PRESENCE OF LIVER ESTERASE (Exp. 4)

$t_{obs.}$ min	x m.equiv. NaOH added	a %	$t_{calc.}$ min
1.35	0.096	5.2	1.29
2.83	0.197	10.7	2.70
4.21	0.297	16.2	4.22
5.67	0.398	21.7	5.70
7.28	0.499	27.2	7.32
9.00	0.600	32.7	9.02
10.79	0.701	38.2	10.84
12.84	0.801	43.6	12.76
14.99	0.902	49.1	14.86
17.30	1.003	54.6	17.14
19.90	1,104	60.0	19.66
22.75	1.205	65.6	22.48
29.73	1.406	76.5	29.50
34.05	1.507	82.0	34.21
39.31	1.608	87.5	40.49
46.40	1.709	93.0	50.20
50.70	1.759	95.7	58.27

a is computed as the ratio of acid liberated divided by one acid equivalent.
$t_{calc.}$ is computed according to equation (1). A'_{tri} = 16.0; B'_{tri} = 4.5.

References p. 91.

A'_{tri} and B'_{tri} are determined graphically and have been used to calculate by equation (1) the time, $t_{calc.}$, corresponding to each observed value of x. a = molarity of tri. The results show close agreement with the observed times, $t_{obs.}$, until about 80% hydrolysis. Then $t_{calc.}$ is always larger than $t_{obs.}$, and the difference is increasing. This is no doubt due to the influence of the next step in the hydrolysis of tripropionyl glycerol (di → mo).

Table II shows that with a constant amount of enzyme, the value of A'_{tri} increases linearly with the initial substrate concentration (a), whereas the value of B'_{tri} remains constant. The experimental results thus agree with equation (1) derived from the reaction scheme suggested for monocaprylate and liver esterase, in which a reversible and an irreversible step is assumed.

TABLE II

EFFECT OF SUBSTRATE CONCENTRATION (a) ON A'_{tri}, B'_{tri} AND $1/v_0$ TRIPROPIONYL GLYCEROL

Exp. no.	*a mmol per litre*	A'_{tri}	B'_{tri}	$1/v_0 = \frac{A'_{tri}}{a} + B'_{tri}$
1	0.460	14.5	4.5	36.0
2	0.919	15.2	4.5	21.1
3*	1.685	15.5	4.5	13.7
4	1.838	16.0	4.5	13.2
5*	3.640	18.2	4.5	9.5

* Exps. 3 and 5 are carried out with other enzyme concentrations than in Exps. 1, 2 and 4. As there is proportionality between initial velocity, v_0, and amount of enzyme, and A'_{tri}/B'_{tri} varies linearly with a, all the experiments could be worked together.

From Fig. 1$_1$ it appears that $1/v_0$ varies linearly with $1/a$; abscissa intercept = k_1/k_2 = 0.405 (determined by the method of least squares).

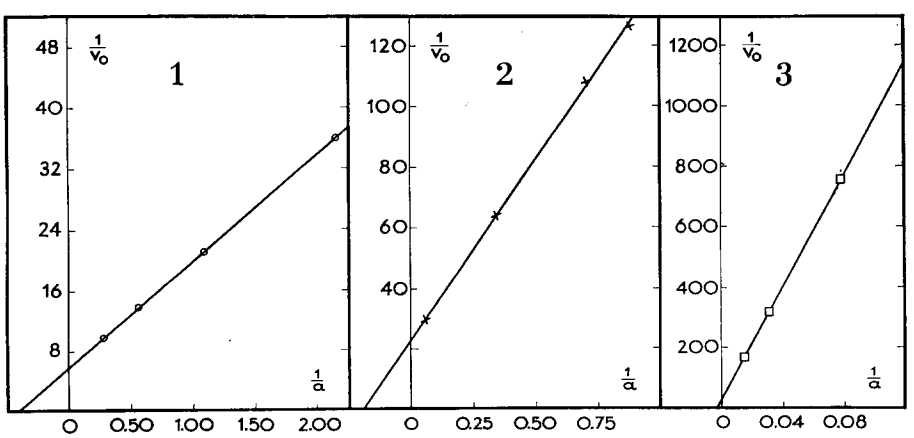

Fig. 1. Influence of initial substrate concentration, a, upon initial velocity, v_0. Abscissa $1/a$ = mmol substrate^{-1}·l. Ordinate $1/v_0$ = m.equiv. base^{-1}·l·min.

1. Tripropionyl glycerol. Abscissa intercept = k_1/k_2 = 0.405.
2. 1,2-dipropionyl glycerol. Abscissa intercept = k_3/k_4 = 0.189.
3. 2-monopropionyl glycerol. Abscissa intercept = k_5/k_6 = 0.00245.

References p. 91.

Kinetics of the reaction: 1,2-dipropionyl glycerol → 2-monopropionyl glycerol + propionic acid (di → mo)

Details for one of the experiments with di are given in Table III. The values for $t_{calc.}$ obtained from equation (1) show close agreement with observed time, $t_{obs.}$, until about 80% hydrolysis of one acid equivalent.

The results obtained in a series of experiments with di are summarized in Table IV. Apparently the action of liver esterase upon di can be explained by the same reaction mechanism as given for liver esterase and tri, putting a in equation (1) equal to molarity of di. A'_{di} varies linearly with a, whereas B'_{di} is constant. Fig 1$_2$ shows a linear relationship between $1/v_0$ and $1/a$; abscissa intercept $= k_3/k_4 = 0.189$.

TABLE III

HYDROLYSIS OF 1,2-DIPROPIONYL GLYCEROL (2.855 mmol per litre) IN THE PRESENCE OF LIVER ESTERASE (Exp. 8)

$t_{obs.}$ *min*	x *m.equiv. NaOH added*	a %	$t_{calc.}$ *min*
7.27	0.114	4.0	7.37
13.80	0.213	7.5	13.91
19.95	0.312	10.9	20.84
26.86	0.412	14.4	27.93
34.33	0.511	17.9	35.25
42.33	0.610	21.4	42.85
50.65	0.709	24.8	50.76
59.68	0.803	28.2	58.92
68.58	0.907	31.8	67.41
77.75	1.006	35.2	76.39
87.65	1.106	38.7	85.82
98.52	1.205	42.2	95.74
109.20	1.304	45.7	106.20
120.56	1.403	49.1	117.29
132.20	1.503	52.6	129.25
144.85	1.601	56.1	141.88
158.20	1.701	59.6	155.49
172.65	1.800	63.1	170.20
187.60	1.899	66.5	186.28
203.78	1.998	70.0	204.01
221.55	2.097	73.5	223.80
242.90	2.196	76.9	246.21
267.20	2.296	80.4	272.15

a is computed as the ratio of acid liberated divided by one acid equivalent. $t_{calc.}$ is computed according to equation (1). $A'_{di} = 153$; $B'_{di} = 10$.

TABLE IV

EFFECT OF SUBSTRATE CONCENTRATION (a) ON A'_{di}, B'_{di} AND $1/v_0$ 1,2-DIPROPIONYL GLYCEROL

Exp. no.	a *mmol per litre*	A'_{di}	B'_{di}	$1/v_0 = \frac{A'_{di}}{a} + B'_{di}$
6	1.136	132	10	126.2
7	1.410	139	10	108.6
8	2.855	153	10	63.6
9	18.11	346	10	29.1

References p. 91.

Kinetics of the reaction: 2-monopropionyl glycerol → glycerol + propionic acid (mo → gl)

Liver esterase has only a very slight activity towards this ester. It is therefore practically impossible in experiments with this ester to reach the degree of hydrolysis which might enable us to calculate some A'_{mo} and B'_{mo} values. For this reason we were content to determine v_0 for three different substrate concentrations using the same extremely large amounts of liver esterase. v_0 was determined graphically from the slope of the first part of the hydrolysis curve. The results are given in Table V. From Fig. 1$_3$ it will be apparent that $1/v_0$ varies linearly with $1/a$.

The experimental data for 2-monopropionyl glycerol may thus be interpreted according to the same reaction scheme as in the cases of tri → di and di → mo in the presence of liver esterase. k_5/k_6 is found to be 0.00245.

TABLE V

EFFECT OF SUBSTRATE CONCENTRATION (a) ON $1/v_0$
2-MONOPROPIONYL GLYCEROL

Exp. no.	*a mmol per litre*	$1/v_0$*
10	12.74	753.6
11	31.87	316.2
12	64.98	166.2

* v_0 is determined graphically from the slope of the first part of the reaction curve.

Calculation of the reaction velocity constants for the processes tri → di, di → mo and mo → gl at the same concentration of liver esterase

As previously described[4] it is a simple matter to compute k_1, k_{-1} and k_2 when the experimental data satisfy equation (1) and A and B have been determined. When E is put equal to 1 and B is known there will be sufficient equations to determine the constants. If B is not known one can only calculate k_1 and k_2, *cf.* equations (2), (3) and (4).

Using the results in Table II and putting $E = 1$ the following k-values are found for the process tri → di:

$$k_1 = 0.0718 \text{ min}^{-1}\text{mmol}^{-1}*$$
$$k_{-1} = 0.01455 \text{ min}^{-1}\text{mmol}^{-1}$$
$$k_2 = 0.1772 \text{ min}^{-1}$$

On the basis of the experiments in Table IV we determined the ratios between the k-values for the process di → mo:

$$k_3 : k_{-3} : k_4 = 0.0840 : 0.00466 : 0.04454$$

These ratios can be used in the calculation of k_3, k_{-3} and k_4 corresponding to the amount of enzyme used in the experiments with tri. It is only necessary to carry out some experiments with tri and di using the same amount of enzyme (E'), *cf.* Table VI.

* In giving this dimension—as in the outlining of the equations which lead to (1)—OH^- is left out of consideration.

References p. 91.

TABLE VI

DETERMINATION OF $1/v_0$ FOR THE SAME CONCENTRATION OF TRIPROPIONYL GLYCEROL AND 1,2-DIPROPIONYL GLYCEROL IN THE PRESENCE OF THE SAME ENZYME CONCENTRATION (E')

Ester	a *mmol per litre*	v_0 *m.equiv./min per litre*	$1/a$	$1/v_0$
Tripropionyl glycerol	1.912	0.0365	0.523	27.4
1,2-dipropionyl glycerol	1.912	0.00269	0.523	372

According to equation (4), using $k_1/k_2 = 0.405$ and $k_3/k_4 = 0.189$ we get

$$E'/v_{0\,(tri)} = 1/k_2 + 0.523/k_1 = 0.928/k_1$$
$$E'/v_{0\,(di)} = 1/k_4 + 0.523/k_3 = 0.712/k_3$$

By division of these two equations we obtain $k_3/k_1 = 0.0565$. From this fraction and $k_{-3}/k_4 = 0.1046$ we finally compute

$$k_3 = 0.004056 \text{ min}^{-1}\text{mmol}^{-1}$$
$$k_{-3} = 0.002245 \text{ min}^{-1}\text{mmol}^{-1}$$
$$k_4 = 0.02146 \text{ min}^{-1}$$

The ratios found between k_1, k_{-1} and k_2 on one side and k_3, k_{-3} and k_4 on the other side have been confirmed by calculations on the basis of other experiments.

In a similar way k_5 and k_6 were computed from experiments with mo and tri using the same concentration of enzyme.

$$k_5 = 0.000286 \text{ min}^{-1}\text{mmol}^{-1}$$
$$k_6 = 0.1167 \text{ min}^{-1}$$

As B'_{mo} has not been determined k_{-5} can not be calculated.

Attempt to treat the hydrolysis of tripropionyl glycerol as a consecutive series of reactions

We will now utilize the information obtained in studies on the separate esters and put forward a reaction scheme for the complete hydrolysis of tripropionyl glycerol in the presence of liver esterase.

$$\left.\begin{array}{lllll} \underset{a-x}{OH^-} + \underset{x}{tri} + \underset{x_1}{X_1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{x_2}{X_2} + \underset{x+y+z}{P^-} & & & & (\pm 1) \\ & \underset{x_2}{X_2} \xrightarrow{k_2} \underset{x_1}{X_1} + \underset{x-y}{di} & & & (+2) \end{array}\right\} \text{I}$$

$$\left.\begin{array}{lllll} \underset{x-y}{OH^-} + \underset{x}{di} + \underset{x_1}{X_1} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \underset{x_3}{X_3} + \underset{x+y+z}{P^-} & & & & (\pm 3) \\ & \underset{x_3}{X_3} \xrightarrow{k_4} \underset{x_1}{X_1} + \underset{y-z}{mo} & & & (+4) \end{array}\right\} \text{II}$$

References p. 91.

$$\left.\begin{array}{l} \underset{y-z}{OH^-} + \underset{x_1}{mo} + \underset{x_4}{X_1} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \underset{x+y+z}{X_4} + P^- \qquad (\pm 5) \\ \underset{x_4}{X_4} \xrightarrow{k_6} \underset{x_1}{X_1} + \underset{z}{gl} \qquad (+6) \end{array}\right\} \text{III}$$

X_1, X_2, X_3 and X_4 are different forms of liver esterase. At time t x_1, x_2, x_3 and x_4 are concentrations of X_1, X_2 etc., x = the molar concentration of hydrolyzed tri; y = molar concentration of hydrolyzed di and z = molar concentration of hydrolyzed mo. a = initial molar concentration of tri. gl = glycerol. The concentration of propionyl ion (P^-) is assumed to be 0 at $t = 0$. The concentration of the hydroxyl ion is constant and does not enter in the mathematical treatment.

When each of the reactions (I), (II) and (III) is supposed to be in the stationary state, *i.e.*

$$\frac{dx_i}{dt} \approx 0 \qquad (i = 2, 3, 4)$$

the rates (using CHRISTIANSEN's notations[5]) are

$$\frac{dx}{dt} = \omega_1 x_1 - \omega_{-1} x_2 \quad (1a) \qquad \omega_1 = k_1 (a - x);\ \omega_{-1} = k_{-1} (x + y + z)$$

$$\frac{dx}{dt} = \omega_2 x_2 \quad (1b) \qquad \omega_2 = k_2$$

$$\frac{dy}{dt} = \omega_3 x_1 - \omega_{-3} x_3 \quad (2a) \qquad \omega_3 = k_3 (x - y);\ \omega_{-3} = k_{-3} (x + y + z)$$

$$\frac{dy}{dt} = \omega_4 x_3 \quad (2b) \qquad \omega_4 = k_4$$

$$\frac{dz}{dt} = \omega_5 x_1 - \omega_{-5} x_4 \quad (3a) \qquad \omega_5 = k_5 (y - z);\ \omega_{-5} = k_{-5} (x + y + z)$$

$$\frac{dz}{dt} = \omega_6 x_4 \quad (3b) \qquad \omega_6 = k_6$$

ω_i is a probability factor and k_i the velocity constant of reaction (i).

Solving (1a) — (3b) for x_2, x_3 and x_4 we have

$$x_2 = \frac{\omega_1}{\omega_{-1} + \omega_2} x_1 \qquad (5a)$$

$$x_3 = \frac{\omega_3}{\omega_{-3} + \omega_4} x_1 \qquad (5b)$$

$$x_4 = \frac{\omega_5}{\omega_{-5} + \omega_6} x_1 \qquad (5c)$$

Introducing x_2, x_3 and x_4 into

$$E = x_1 + x_2 + x_3 + x_4 \qquad (6)$$

we get

$$E = \left(1 + \frac{\omega_1}{\omega_{-1} + \omega_2} + \frac{\omega_3}{\omega_{-3} + \omega_4} + \frac{\omega_5}{\omega_{-5} + \omega_6}\right) x_1 \qquad (7)$$

References p. 91.

From (1a) and (1b)

$$x_1 = \frac{\omega_{-1} + \omega_2}{\omega_1 \omega_2} \frac{dx}{dt}$$

from which with equation (7)

$$E\frac{dt}{dx} = \frac{1}{\omega_1 \omega_2}\left(\omega_2 + \omega_{-1} + \omega_1 + \omega_3 \frac{\omega_{-1} + \omega_2}{\omega_{-3} + \omega_4} + \omega_5 \frac{\omega_{-1} + \omega_2}{\omega_{-5} + \omega_6}\right) \tag{8a}$$

Inserting the probability factor ω_i in (8a), which is analogous with equation (5) in Paper I[3] in this series, we get

$$E\frac{dt}{dx} = \frac{1}{k_1}\left[\frac{k_1}{k_2} + \frac{1}{a-x} + \frac{k_{-1}}{k_2}\frac{x+y+z}{a-x} + \frac{k_3}{k_2}\frac{x-y}{a-x}\frac{k_{-1}(x+y+z)+k_2}{k_{-3}(x+y+z)+k_4} + \frac{k_5}{k_2}\frac{y-z}{a-x}\frac{k_{-1}(x+y+z)+k_2}{k_{-5}(x+y+z)+k_6}\right] \tag{8b}$$

For $t = 0$, when $x = y = z = 0$, equation (8b) reduces to

$$\frac{E}{v_0} = \frac{1}{k_2} + \frac{1}{k_1}\frac{1}{a}$$

where $v_0 = \left[\frac{dx}{dt}\right]_{t=0} = \left[\frac{d(x+y+z)}{dt}\right]_{t=0}$

It will be very difficult to integrate equation (8b) unless $k_{-1}/k_2 = k_{-3}/k_4 = k_{-5}/k_6$ or these ratios are of the same order of magnitude. From the calculations on p. 80 it appears that $k_{-3}/k_4 = 0.1046$ which agrees fairly well with $k_{-1}/k_2 = 0.0821$. The agreement demonstrated is sufficiently good to justify the following simplification of (8b) to (9). As working hypothesis we also assume that k_{-5}/k_6, which could not be determined, is of the order of magnitude of 0.1.

$$E\frac{dt}{dx} = \frac{1}{k_1}\left(\frac{k_1}{k_2} + \frac{1}{a-x} + \frac{k_{-1}}{k_2}\frac{x+y+z}{a-x} + \frac{k_3}{k_4}\frac{x-y}{a-x} + \frac{k_5}{k_6}\frac{y-z}{a-x}\right) \tag{9}$$

Substituting $x + y + z = 3a - 3(a-x) - 2(x-y) - (y-z)$ in equation (9) we get

$$E\frac{dt}{dx} = \frac{1}{k_1}\left(1 + 3a\frac{k_{-1}}{k_2}\right)\frac{1}{a-x} + \frac{1}{k_2}\left(1 - 3\frac{k_{-1}}{k_1}\right) + \frac{1}{k_1}\left(\frac{k_3}{k_4} - 2\frac{k_{-1}}{k_2}\right)\frac{x-y}{a-x} + \frac{1}{k_1}\left(\frac{k_5}{k_6} - \frac{k_{-1}}{k_2}\right)\frac{y-z}{a-x} \tag{10}$$

In order to integrate equation (10), $\frac{y-x}{a-x}$ and $\frac{y-z}{a-x}$ must be expressed as functions of x.

From (1b), (2b) and (3b) with (5a), (5b) and (5c) we find

$$\frac{dy}{dx} = \frac{\omega_3 \omega_4}{\omega_1 \omega_2}\frac{\omega_{-1} + \omega_2}{\omega_{-3} + \omega_4}; \qquad \frac{dz}{dx} = \frac{\omega_5 \omega_6}{\omega_1 \omega_2}\frac{\omega_{-1} + \omega_2}{\omega_{-5} + \omega_6}$$

References p. 91.

Insertion of ω_i, putting $k_{-1}/k_2 = k_{-3}/k_4 = k_{-5}/k_6$ leads to

$$\frac{dy}{dx} = k_{\mathrm{I}} \frac{x-y}{a-x} \tag{11}$$

and

$$\frac{dz}{dx} = k_{\mathrm{II}} \frac{y-z}{a-x} \tag{12}$$

with $k_{\mathrm{I}} = k_3/k_1$ and $k_{\mathrm{II}} = k_5/k_1$.

Equation (11) can be integrated to yield

$$y = a \left\{ 1 - \frac{1}{1-k_{\mathrm{I}}} \left[\left(\frac{a-x}{a} \right)^{k_{\mathrm{I}}} - k_1 \frac{a-x}{a} \right] \right\} \tag{13}$$

hence

$$\frac{x-y}{a-x} = \frac{1}{1-k_{\mathrm{I}}} \left[\left(\frac{a-x}{a} \right)^{k_{\mathrm{I}}-1} - 1 \right] \tag{14}$$

Equation (12) can be integrated using equation (13) to yield

$$z = a \left\{ 1 + \frac{k_{\mathrm{II}}}{(1-k_1)(k_{\mathrm{I}}-k_{\mathrm{II}})} \left(\frac{a-x}{a} \right)^{k_{\mathrm{I}}} - \frac{k_{\mathrm{I}}}{(1-k_{\mathrm{II}})(k_{\mathrm{I}}-k_{\mathrm{II}})} \left(\frac{a-x}{a} \right)^{k_{\mathrm{II}}} - \frac{k_{\mathrm{I}} k_{\mathrm{II}}}{(1-k_1)(1-k_{\mathrm{II}})} \frac{a-x}{a} \right\} \tag{15}$$

Hence

$$\frac{y-z}{a-x} = \frac{k_{\mathrm{I}}}{(1-k_{\mathrm{II}})(k_{\mathrm{I}}-k_{\mathrm{II}})} \left(\frac{a-x}{a} \right)^{k_{\mathrm{II}}-1} - \frac{k_{\mathrm{I}}}{(1-k_1)(k_{\mathrm{I}}-k_{\mathrm{II}})} \left(\frac{a-x}{a} \right)^{k_{\mathrm{I}}-1} + \frac{k_{\mathrm{I}}}{(1-k_1)(1-k_{\mathrm{II}})} \tag{16}$$

Introduction of (14) and (16) into (10) leads to

$$E\frac{dt}{dx} = \frac{1}{k_1}\left(1 + 3a\frac{k_{-1}}{k_2}\right)\frac{1}{a-x} + \frac{1}{k_2}\left(1 - 3\frac{k_{-1}}{k_1}\right) - \frac{1}{k_1}\frac{1}{1-k_{\mathrm{I}}}\left(\frac{k_3}{k_4} - 2\frac{k_{-1}}{k_2}\right) + \frac{1}{k_1}\frac{k_{\mathrm{I}}}{(1-k_1)(1-k_{\mathrm{II}})}\left(\frac{k_5}{k_6} - \frac{k_{-1}}{k_2}\right) + \frac{1}{k_1}\left[\left(\frac{k_3}{k_4} - 2\cdot\frac{k_{-1}}{k_2}\right)\frac{1}{1-k_{\mathrm{I}}} - \left(\frac{k_5}{k_6} - \frac{k_{-1}}{k_2}\right)\frac{k_{\mathrm{I}}}{(1-k_1)(k_{\mathrm{I}}-k_{\mathrm{II}})}\right]\left(\frac{a-x}{a}\right)^{k_{\mathrm{I}}-1} + \frac{1}{k_1}\left(\frac{k_5}{k_6} - \frac{k_{-1}}{k_2}\right)\frac{k_{\mathrm{I}}}{(1-k_{\mathrm{II}})(k_{\mathrm{I}}-k_{\mathrm{II}})}\left(\frac{a-x}{a}\right)^{k_{\mathrm{II}}-1} \tag{17}$$

Integration of equation (17) yields

$$Et = A \ln\frac{a}{a-x} + Bx + C\left[1 - \left(\frac{a-x}{c}\right)^{k_{\mathrm{I}}}\right] + D\left[1 - \left(\frac{a-x}{a}\right)^{k_{\mathrm{II}}}\right] \tag{18}$$

References p. 91.

in which

$$A = \frac{1}{k_1}\left(1 + 3a\frac{k_{-1}}{k_2}\right)$$

$$B = \frac{1}{k_1}\left[\frac{k_1}{k_2} - 3\frac{k_{-1}}{k_2} - \left(\frac{k_3}{k_4} - 2\frac{k_{-1}}{k_2}\right)\frac{1}{1-k_I} + \left(\frac{k_5}{k_6} - \frac{k_{-1}}{k_2}\right)\frac{k_I}{(1-k_I)(1-k_{II})}\right]$$

$$C = \frac{a}{k_3}\left[\left(\frac{k_3}{k_4} - 2\frac{k_{-1}}{k_2}\right)\frac{1}{1-k_I} - \left(\frac{k_5}{k_6} - \frac{k_{-1}}{k_2}\right)\frac{k_I}{(1-k_I)(k_I-k_{II})}\right]$$

$$D = \frac{a}{k_5}\left(\frac{k_5}{k_6} - \frac{k_{-1}}{k_2}\right)\frac{k_I}{(1-k_{II})(k_I-k_{II})}$$

Equations (13), (15) and (18) form a parameter description of the relationship between added base $(x + y + z)$ and time t, x (the amount of acid liberated in the process tri → di) being the parameter.

It is, however, more convenient to use another parameter, *viz.* $u = \log_{10}\frac{a}{a-x}$, equation (18) being rewritten:

$$t = T\left(F + G + H + I \cdot u \cdot \ln 10 - \frac{F}{10^u} - \frac{G}{10^{k_I u}} - \frac{H}{10^{k_{II} u}}\right) \tag{19}$$

The same parameter is used for the calculation of added base $(x + y + z)$

$$x + y + z = a\left(3 - \frac{K}{10^u} - \frac{L}{10^{k_I u}} - \frac{M}{10^{k_{II} u}}\right) \tag{20}$$

$$F = \frac{k_1}{k_{-1}} - 3 - \left(\frac{k_3}{k_{-3}} - 2\right)\frac{1}{1-k_I} + \left(\frac{k_5}{k_{-5}} - 1\right)\frac{k_I}{(1-k_I)(1-k_{II})}; \qquad (= 2.083)$$

$$G = \frac{1}{k_I}\left(\frac{k_3}{k_{-3}} - 2\right)\frac{1}{1-k_I} - \left(\frac{k_5}{k_{-5}} - 1\right)\frac{1}{(1-k_I)(k_I-k_{II})}; \qquad (= 15.96)$$

$$H = \frac{1}{k_{II}}\left(\frac{k_5}{k_{-5}} - 1\right)\frac{k_I}{(1-k_{II})(k_I-k_{II})}; \qquad (= -263.69)$$

$$F + G + H = -245.647$$

$$K = 1 - \frac{k_I}{1-k_I} + \frac{k_I k_{II}}{(1-k_I)(1-k_{II})}; \qquad (= 0.940)$$

$$L = \frac{1}{1-k_I} - \frac{k_{II}}{(1-k_I)(k_I-k_{II})}; \qquad (= 0.980)$$

$$M = \frac{k_I}{(1-k_{II})(k_I-k_{II})}; \qquad (= 1.082)$$

$$K + L + M = 3.0$$

It is seen that F, G, H, K, L and M do not contain E (enzyme concentration) or a (substrate concentration). These constants may therefore be used in any experiment with tripropionyl glycerol and liver esterase. Their numerical values are computed by means of the numbers given previously for k_1, k_{-1}, k_2, k_3, k_{-3}, k_4, k_5 and k_6. We assume (*cf.* p. 83) $k_{-5}/k_6 = k_{-1}/k_2 = 0.0821$. Then $k_5/k_{-5} = k_5/0.0821k_6$. As previously mentioned $k_I = k_3/k_1 = 0.0565$ and $k_{II} = k_5/k_1 = 0.00398$.

References p. 91.

TABLE VII

HYDROLYSIS OF TRIPROPIONYL GLYCEROL IN THE PRESENCE OF LIVER ESTERASE
EXP. 5. a = 3.640 mmol PER LITRE

$t_{obs.}$ min	$\log_{10} t_{obs.}$	$x+y+z$ m.equiv. NaOH added per l	$t_{obs.}$ min	$\log_{10} t_{obs.}$	$x+y+z$ m.equiv. NaOH added per l
0.69	—0.141	0.160	48.50	1.686	4.044
1.27	0.104	0.284	51.80	1.714	4.106
1.85	0.267	0.407	55.35	1.743	4.168
2.36	0.373	0.530	59.20	1.772	4.229
3.00	0.477	0.653	62.60	1.797	4.291
3.66	0.564	0.777	66.50	1.823	4.352
4.12	0.615	0.900	70.76	1.850	4.414
4.77	0.679	1.023	75.00	1.875	4.476
5.46	0.737	1.147	78.70	1.896	4.537
5.78	0.762	1.208	82.50	1.916	4.599
6.47	0.811	1.332	87.15	1.940	4.661
6.83	0.834	1.393	91.88	1.963	4.772
7.62	0.882	1.517	96.05	1.983	4.784
8.29	0.919	1.640	100.85	2.004	4.846
9.00	0.954	1.763	105.28	2.022	4.907
9.40	0.973	1.825	109.68	2.041	4.969
9.85	0.993	1.886	114.70	2.060	5.031
10.27	1.012	1.948	119.95	2.079	5.092
11.19	1.049	2.071	125.05	2.097	5.154
11.62	1.065	2.133	130.05	2.114	5.216
12.13	1.084	2.195	136.15	2.134	5.277
12.60	1.100	2.256	142.45	2.154	5.339
13.18	1.120	2.318	147.70	2.169	5.401
13.63	1.135	2.380	153.30	2.186	5.462
14.13	1.150	2.441	159.6	2.203	5.524
14.70	1.167	2.503	165.4	2.219	5.585
15.32	1.185	2.565	172.0	2.236	5.647
16.55	1.219	2.688	178.2	2.251	5.709
17.03	1.231	2.750	185.6	2.269	5.770
17.72	1.248	2.811	192.6	2.285	5.832
18.43	1.266	2.873	200.3	2.302	5.894
19.12	1.282	2.935	208.4	2.319	5.955
19.90	1.299	2.996	216.7	2.336	6.017
20.67	1.315	3.056	224.8	2.352	6.079
21.52	1.333	3.119	233.6	2.368	6.140
22.40	1.350	3.181	243.9	2.387	6.202
23.49	1.371	3.243	252.5	2.402	6.264
24.67	1.392	3.304	261.3	2.417	6.325
25.56	1.408	3.366	270.5	2.432	6.387
26.85	1.429	3.428	279.6	2.447	6.449
28.10	1.449	3.489	290.0	2.462	6.510
29.30	1.462	3.551	313.1	2.496	6.634
31.05	1.492	3.613	327.8	2.517	6.757
32.82	1.516	3.674	355.8	2.551	6.818
35.00	1.544	3.736	370.5	2.569	6.880
37.30	1.572	3.798	387.9	2.589	6.942
39.85	1.600	3.859	407.0	2.610	7.004
42.63	1.630	3.921	426.5	2.630	7.065
45.32	1.656	3.983			

References p. 91.

T and I both depend on a, T also on the enzyme concentration

$$T = \frac{k_{-1}}{k_1 k_2} \frac{a}{E}$$

$$I = \frac{1}{a} \frac{k_2}{k_{-1}} + 3$$

An example (Exp. 5) will illustrate that it is possible by means of equations (19) and (20) to render the course of hydrolysis of tripropionyl glycerol in the presence of liver esterase.

Experiment 5

The concentration of tripropionyl glycerol was 3.640 mmol per litre. The hydrolysis was followed through 426½ minutes, when the experiment was stopped because the hydrolysis now proceeded extremely slowly. At this time an amount of acid corresponding to 3.64 × 1.94 milliequivalents per litre had been liberated. In Table VII are given corresponding values of time ($t_{obs.}$) and base added to neutralize acid liberated. For practical reasons (see later) $\log_{10} t_{obs.}$ is also listed.

TABLE VIII

CALCULATION OF $x+y+z$ ACCORDING TO EQUATION (20)
(EXP. 5)

u	$k_I u$	$k_{II} u$	10^u	$10^{k_I u}$	$10^{k_{II} u}$	$K/10^u$	$L/10^{k_I u}$	$M/10^{k_{II} u}$	$\frac{x+y+z}{a}$	$x+y+z$
0	0	0	1.0000	1.0000	1.0000	0.940	0.980	1.082	0	0
0.025	0.00141	0.00010	1.0592	1.0032	1.0002	0.887	0.977	1.082	0.056	0.204
0.050	0.00283	0.00020	1.1220	1.0065	1.0005	0.838	0.973	1.082	0.109	0.397
0.075	0.00424	0.00030	1.1885	1.0098	1.0007	0.791	0.970	1.081	0.160	0.582
0.10	0.00565	0.00040	1.2589	1.0131	1.0009	0.747	0.966	1.081	0.204	0.757
0.14	0.00791	0.00056	1.3804	1.0184	1.0013	0.681	0.963	1.081	0.277	1.008
0.20	0.01130	0.00080	1.5849	1.0264	1.0017	0.593	0.954	1.080	0.375	1.365
0.25	0.01413	0.00100	1.7783	1.0331	1.0023	0.529	0.949	1.080	0.444	1.616
0.30	0.0170	0.0012	1.995	1.040	1.002	0.471	0.942	1.080	0.509	1.853
0.40	0.0226	0.0016	2.512	1.053	1.003	0.374	0.930	1.079	0.617	2.253
0.50	0.0283	0.0020	3.162	1.068	1.005	0.297	0.918	1.077	0.700	2.548
0.60	0.0339	0.0024	3.981	1.081	1.006	0.236	0.907	1.076	0.783	2.850
0.70	0.0396	0.0028	5.012	1.095	1.007	0.188	0.895	1.074	0.845	3.076
0.80	0.0452	0.0032	6.310	1.109	1.007	0.149	0.884	1.074	0.895	3.258
0.90	0.0509	0.0036	7.943	1.124	1.008	0.118	0.872	1.073	0.939	3.418
1.00	0.0565	0.0040	10.000	1.139	1.009	0.094	0.860	1.072	0.976	3.553
1.50	0.0848	0.0060	31.62	1.216	1.014	0.030	0.806	1.067	1.099	4.000
2.00	0.1131	0.0080	10^2	1.297	1.019	0.009	0.756	1.062	1.175	4.277
3.00	0.1695	0.0119	10^3	1.477	1.028	0.001	0.664	1.053	1.284	4.674
4.00	0.2260	0.0159	10^4	1.683	1.037	0	0.582	1.043	1.377	5.012
5.00	0.2825	0.0199	10^5	1.916	1.047	0	0.511	1.033	1.458	5.307
6.00	0.3390	0.0239	10^6	2.183	1.056	0	0.449	1.024	1.529	5.566
7.00	0.3955	0.0279	10^7	2.486	1.066	0	0.394	1.015	1.593	5.799
8.00	0.4520	0.0318	10^8	2.831	1.076	0	0.346	1.006	1.650	6.006
9.00	0.5085	0.0358	10^9	3.225	1.086	0	0.304	0.996	1.702	6.195
10.00	0.5650	0.0398	10^{10}	3.673	1.096	0	0.267	0.987	1.748	6.363
12.00	0.6780	0.0478	10^{12}	4.764	1.116	0	0.206	0.970	1.826	6.647
14.00	0.7910	0.0557	10^{14}	6.180	1.137	0	0.159	0.952	1.891	6.883
16.00	0.9040	0.0637	10^{16}	8.017	1.157	0	0.122	0.935	1.945	7.080
18.00	1.0170	0.0716	10^{18}	10.40	1.179	0	0.094	0.918	1.990	7.244

References p. 91.

TABLE IX

CALCULATION OF t ACCORDING TO EQUATION (19) (EXP. 5)

u	$Iu \cdot ln\ 10$	$F/10^u$	$G/10^{k_{II}u}$	$H/10^{k_{III}u}$	t/T	$t_{calc.}$	$log_{10}t_{calc.}$
0	0	2.083	15.960	263.690	0	0	
0.025	0.365	1.967	15.909	263.637	0.479	0.90	—0.046
0.050	0.730	1.856	15.857	263.558	0.927	1.75	0.243
0.075	1.095	1.753	15.805	263.506	1.396	2.63	0.420
0.10	1.460	1.655	15.754	263.453	1.857	3.50	0.544
0.14	2.044	1.509	15.672	263.348	2.564	4.84	0.685
0.20	2.920	1.314	15.549	263.248	3.652	6.89	0.838
0.25	3.650	1.171	15.449	263.085	4.468	8.43	0.926
0.30	4.38	1.04	15.35	263.16	5.51	10.39	1.017
0.40	5.84	0.83	15.16	262.90	7.11	13.41	1.127
0.50	7.30	0.66	14.94	262.38	8.44	15.92	1.202
0.60	8.76	0.52	14.76	262.12	9.96	18.78	1.274
0.70	10.22	0.42	14.58	261.86	11.44	21.58	1.334
0.80	11.68	0.33	14.39	261.86	13.18	24.86	1.396
0.90	13.14	0.26	14.20	261.60	14.64	27.61	1.441
1.00	14.60	0.21	14.01	261.34	16.08	30.33	1.482
1.50	21.90	0.07	13.13	260.05	23.11	43.59	1.639
2.00	29.20	0.02	12.31	258.77	30.00	56.58	1.752
3.00	43.80	0	10.81	256.51	43.86	82.72	1.918
4.00	58.40	0	9.48	254.28	57.56	108.6	2.036
5.00	73.00	0	8.33	251.85	70.88	136.7	2.136
6.00	87.60	0	7.31	249.71	84.35	151.1	2.202
7.00	102.20	0	6.42	247.36	97.50	183.9	2.265
8.00	116.80	0	5.64	245.07	110.59	208.6	2.319
9.00	131.40	0	4.95	242.81	123.62	233.2	2.368
10.00	146.00	0	4.35	240.59	136.60	257.6	2.411
12.00	175.20	0	3.35	236.28	168.49	306.5	2.487
14.00	204.40	0	2.58	231.92	188.10	354.8	2.550
16.00	233.60	0	1.99	227.91	213.97	403.5	2.606
18.00	262.80	0	1.53	223.66	239.28	451.3	2.655

The calculation of t and $x + y + z$ according to equations (19) and (20) was carried out as follows. The parameter $u = \log_{10} \frac{a}{a - x}$ was first inserted in equation (20) and $x + y + z$ was computed, *cf.* Table VIII. Then t was calculated according to equation (19) using the values of 10^u, $10^{k_{II}u}$ and $10^{k_{III}u}$, which are recorded in Table VIII, together with the values of F, G, H already mentioned, *cf.* Table IX. In the calculation of t from equation (19) it is also necessary to know the numerical values of T and I. In Exp. 5 a is equal to 3.640, hence $I = \frac{1}{a} \frac{k_2}{k_{-1}} + 3 = 6.34$. The enzyme concentration in the experiment mentioned is not known. However, in the *beginning* of any hydrolysis experiment with tripropionyl glycerol and liver esterase the equations (1), (2) and (3) are valid. On the basis of the experimental data we determine graphically

$$B'_{tri} = \frac{B_{tri}}{E} = \frac{1}{Ek_2}\left(1 - \frac{k_{-1}}{k_1}\right) = 2.04$$

and find

$$T = \frac{k_{-1}}{k_1} \frac{a}{Ek_2} = 1.886$$

References p. 91.

In Fig. 2 log $t_{calc.}$ from equation (19), *cf.* Table IX, and log $t_{obs.}$, *cf.* Table VII, are plotted against added base in m.equivs. per litre $(x + y + z)$. The points obtained from the experiment lie closely on the same curve as those obtained by the calculations. The difference between log $t_{calc.}$ and log $t_{obs.}$ for a given value of $x + y + z$ is less than 0.04, usually 0.01—0.02, which means that the observed t values deviate only a few per cent from the calculated.

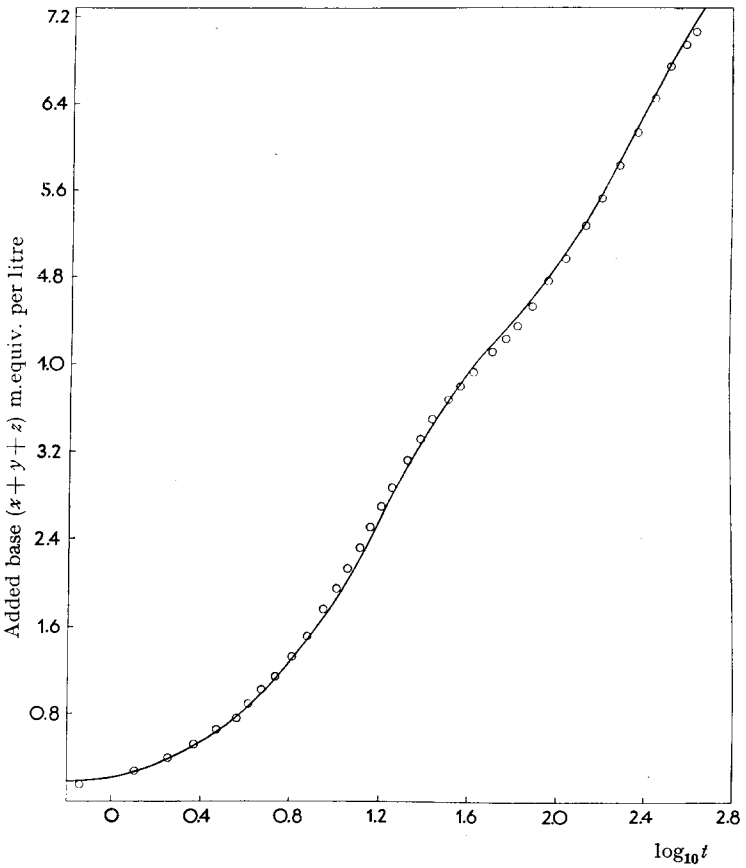

Fig. 2. Exp. 5. Comparison between experimental values of time t and base added (*cf.* Table VII and calculated values obtained from equations (19) and (20). The curve is drawn through calculated values (*cf.* Tables VIII and IX). o = experimental values.

DISCUSSION

In the range of hydrolysis which has been studied the experimental findings agree with the equations derived from the reaction scheme suggested. It appears from Table VII that in Exp. 5 about 65% of the total acid contained in the substrate has been liberated. At this time one is able to calculate from equations (13), (15) and Table VIII that $x/a = 1.00$, $y/a = 0.865$ and $z/a = 0.075$. It might have been of interest to attain a higher degree of hydrolysis than reached. It should, however, be noted that in order to proceed further within a reasonable time it is necessary to work with such large con-

References p. 91.

centrations of enzyme that it will be impossible, by means of the technique employed, to determine the beginning of the hydrolysis curve with accuracy. If this part of the curve is not well defined by help of several observations no exact calculation can be carried out. Furthermore, it is clear that we have followed almost completely that part of the hydrolysis process which kinetically must be most difficult to interpret. The reaction mo —> gl has already been examined by using 2-monopropionyl glycerol as substrate.

It is not of course possible to conclude that the reaction mechanism suggested is the only possible way of explaining the hydrolysis of tripropionyl glycerol by liver esterase. Apparently the reaction scheme put forward is the simplest possible, allowing a clear mathematical treatment. Each separate step in the process consists of a minimum of partial reactions. If the reaction were treated in a more traditional manner it would be necessary to assume that in each of the steps entered the formation of an inactive enzyme-propionate complex. This would impede the mathematical treatment. The manner of proceeding outlined for the kinetic problem here may be of interest in connection with other enzymatic consecutive reactions.

ACKNOWLEDGEMENT

This work has been aided by a grant from "Carlsberg Foundation".

SUMMARY

The kinetics of the hydrolysis of tripropionyl glycerol by liver esterase—a reaction which according to previously published investigations proceeds via 1,2-dipropionyl and 2-monopropionyl glycerol—have been made the subject of a closer examination.

1. The experimental findings justify the assumption that the transformation in each of the reactions tripropionyl glycerol to 1,2-dipropionyl glycerol + propionic acid, 1,2-dipropionyl glycerol to 2-monopropionyl glycerol + propionic acid and 2-monopropionyl glycerol to glycerol and propionic acid is explained by a reaction scheme which is a closed sequence consisting of two partial reactions. On the basis of experiments in which the initial substrate concentration is varied the following k-values are calculated for one and the same enzyme concentration ($E = 1$): $k_1 = 0.0718$ $min^{-1}mmol^{-1}$, $k_{-1} = 0.01455$ $min^{-1}mmol^{-1}$, $k_2 = 0.1772$ min^{-1}, $k_3 = 0.004056$ $min^{-1}mmol^{-1}$, $k_{-3} = 0.002245$ $min^{-1}mmol^{-1}$, $k_4 = 0.02146$ min^{-1}, $k_5 = 0.000286$ $min^{-1}mmol^{-1}$, $k_6 = 0.1167$ min^{-1}.

2. A conceivable reaction scheme is put forward for the complete hydrolysis of tripropionyl glycerol by liver esterase, *cf.* p. 81. One and the same enzyme is supposed to catalyze the degradation of tripropionyl glycerol and the intermediate glycerides. Each of the three consecutive reactions is assumed to consist of two partial reactions which are in a stationary state. On the assumption that $k_{-1}/k_2 = k_{-3}/k_4 = k_{-5}/k_6$ or that the ratios are of the same order of magnitude equations are derived which using $\log_{10} \frac{a}{a-x}$ as a parameter are able to render the experimental data quite satisfactorily.

3. The mathematical treatment of the kinetic problem investigated may be of interest in connection with other enzymatic consecutive reactions.

RÉSUMÉ

Nous avons soumis à un examen approfondi la cinétique de l'hydrolyse du tripropionyl glycérol par l'action de l'estérase hépatique une réaction qui, selon nos investigations précedentes, passe par le 1,2-dipropionyl glycérol et le 2-monopropionyl glycérol.

1. Les découvertes expérimentales justifient la supposition que la transformation du tripropionyl glycérol en 1,2-dipropionyl glycérol et acide propionique, du 1,2-dipropionyl glycérol en 2-monopropionyl glycérol et acide propionique et du 2-monopropionyl glycérol en glycérol et acide propionique, peut être expliquée par un mécanisme de réaction correspondant à un cycle composé de 2 réactions partielles. Sur la base d'expériences au cours desquelles l'on fait varier la concentration

References p. 91.

initiale du substrat nous pouvons calculer (en posant la quantité d'enzyme = 1): $k_1 = 0.0718$ min^{-1} $mmol^{-1}$, $k_{-1} = 0.01455$ $min^{-1}mmol^{-1}$, $k_2 = 0.1772$ min^{-1}, $k_3 = 0.004056$ $min^{-1}mmol^{-1}$, $k_{-3} = 0.002245$ $min^{-1}mmol^{-1}$, $k_4 = 0.02146$ min^{-1}, $k_5 = 0.000286$ $min^{-1}mmol^{-1}$, $k_6 = 0.1167$ min^{-1}.

2. Nous proposons un schéma de réaction pour l'hydrolyse totale du tripropionyl glycerol par l'estérase hépatique, *cf.* p. 81. Nous supposons qu'une seule et même enzyme catalyse la dégradation du tripropionyl glycérol et des glycérides partiels intermédiaires. Nous postulons que chacune des réactions consécutives est composée de 2 réactions partielles qui sont à l'état stationnaire. En supposant que $k_{-1}/k_2 = k_{-3}/k_4 = k_{5-}/k_6$ ou que ces proportions sont du même ordre de grandeur, nous déduisons des équations qui, en employant $\log_{10} \frac{a}{a-x}$ comme paramètre, s'accordent de mnière satisfaisante avec les données expérimentales.

3. Le traitement mathématique du problème cinétique étudié pourrait être intéressant en relation avec d'autres réactions consécutives catalysées par des enzymes.

ZUSAMMENFASSUNG

Die Kinetik der Hydrolyse von Tripropionylglycerol durch Leberesterase—ein Prozess der nach früheren Untersuchungen über 1,2-Dipropionylglycerol und 2-Monopropionylglycerol verläuft—wurde zum Gegenstand einer genaueren Untersuchung gemacht.

2. Unsere Versuche rechtfertigen die Annahme, dass der Abbau von Tripropionylglycerol zu 1,2-Dipropionylglycerol + Propionsäure, von 1,2-Dipropionylglycerol zu 2-Monopropionylglycerol + Propionsäure und von 2-Monopropionylglycerol zu Glycerol + Propionsäure nach einem Reaktionsmechanismus verläuft, der eine geschlossene aus 2 Teilreaktionen bestehende Kette ist. Auf Grund von Versuchen, in welchen die Anfangskonzentration geändert wird, kann man für dieselbe Enzymkonzentration ($E = 1$) die folgenden k-Werte berechnen: $k_1 = 0.0718$ $min^{-1}mmol^{-1}$, $k_{-1} = 0.01455$ $min^{-1}mmol^{-1}$, $k_2 = 0.1772$ min^{-1}, $k_3 = 0.004056$ $min^{-1}mmol^{-1}$, $k_{-3} = 0.002245$ $min^{-1}mmol^{-1}$, $k_4 = 0.02146$ min^{-1}, $k_5 = 0.000286$ $min^{-1}mmol^{-1}$, $k_6 = 0.1167$ min^{-1}.

2. Ein Reaktionsmechanismus für die vollständige Hydrolyse von Tripropionylglycerol durch Leberesterase wird aufgestellt, vgl. S. 81. Es wird angenommen, dass dasselbe Enzym beim Abbau des Tripropionylglycerols und in den nachfolgenden Stufen wirksam ist. Jede der drei auf einander folgenden Stufen besteht aus 2 Teilreaktionen, die in stationärem Zustand sind. Nimmt man an, dass $k_{-1}/k_2 = k_{-3}/k_4 = k_{-5}/k_6$, oder dass diese Proportionen von gleicher Grössenordnung sind, so kann man Gleichungen ableiten, die unter Verwendung des Parameters $\log_{10} \frac{a}{a-x}$ in befriedigender Weise die Versuchsergebnisse wiedergeben können.

3. Die mathematische Behandlung des untersuchten kinetischen Problems kann auch in Verbindung mit anderen enzymatischen Reaktionsfolgen von Interesse sein.

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Received September 15th, 1951