THEORETICAL INTERPRETATION OF ACETOACETATE FORMATION FROM SHORT-CHAIN FATTY ACIDS OF THE EVEN SERIES†

by

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Recent isotope work has demonstrated that fatty acids in liver and kidney preparations are degraded to two-carbon (C_2) fragments which may then condense to form acetoacetic acid (AAA) and/or be oxidized to CO_2 via the tricarboxylic acid cycle[1-13]. Even-carbon fatty acids containing ^{13}C or ^{14}C in any odd carbon (carboxyl, β, δ, etc.) form AAA in which the isotope resides only in the carbonyl and carboxyl carbons[9,10]. WEINHOUSE, MEDES, AND FLOYD[1] first observed random condensation of C_2 fragments from carboxyl-labelled octanoate. However, subsequent work indicates that, in general, isotopic C_2 fragments from short-chain fatty acids are not equally distributed between the carbonyl and carboxyl moieties of AAA[3,5,6,7,8,11,12].

The relative incorporation of isotopic C_2 fragments into the two moieties of AAA may be represented by the $C^*O:C^*OOH$ ratio. Little attempt has hitherto been made to explain the various $C^*O:C^*OOH$ ratios obtained by different groups of workers except on a qualitative basis. A notable exception to this is the analysis put forth by CRANDALL AND GURIN[5] and CRANDALL, BRADY, AND GURIN[6]. These investigators have proposed that the ratios observed by them for octanoate-1-^{14}C (0.74), and octanoate-7-^{14}C (3.3) are mutually consistent if it be assumed that fatty acids give rise to two types of C_2 fragments, (CH_3CO–) and (–CH_2CO–). According to this concept, the first type is derived from the terminal two carbons distal to the carboxyl group of a fatty acid, and the second is derived from all other potential C_2 fragments of the fatty acid as well as from the partial conversion of (CH_3CO–) $\longrightarrow$ (–CH_2CO–). The (CH_3CO–) type fragments are presumed to be incorporated only into the carbonyl moiety of AAA, while (–CH_2CO–) type fragments can go readily to either moiety.

It has generally been assumed that C_2 fragments from many molecules mix so that the carbonyl and carboxyl moieties of any molecule of AAA can be derived from C_2 fragments from different fatty acid molecules. The condensation of C_2 fragments by this process would be *intermolecular*. Recent observations by BEINERT *et al.*[14] leave open the possibility that C_2 fragments from fatty acids which condense to form AAA may not require the participation of an *intermolecular* condensing enzyme. According to GREEN[15], the results obtained with short-chain fatty acids can be explained on a basis of non-random, *intramolecular* condensation of C_2 fragments on an enzyme surface. We interpret the hypothesis offered by GREEN to mean that the terminal two carbons distal

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to the carboxyl group of an even fatty acid do not differ (other than in position on the enzyme surface) from other potential C_2 fragments of the fatty acid.

The purpose of this paper is to show how the concept of two types of C_2 fragments, as indicated above, lends itself to a theoretical analysis for the distribution of isotope in AAA from short-chain, even-carbon fatty acids (4–12 carbons). In making use, here, of this concept it should not be overlooked, however, that the assumption of two types of C_2 fragments may be nothing but a convenient device to represent a complex, non-random condensation process on an enzyme surface.

I. TERMINOLOGY

(CH_3CO-) = C_2 fragments arising from the terminal two carbons (distal to the carboxyl group) of a fatty acid.

$(-CH_2CO-)$ = C_2 fragments arising from non-terminal carbons of a fatty acid, or from the conversion $(CH_3CO-) \longrightarrow (-CH_2CO-)$.

$(-CH_2C^{*}O-)_i$ = Isotopic C_2 fragments of the $(-CH_2CO-)$ type before dilution by non-isotopic fragments.

$(CH_3C^{*}O-)_i$ = Isotopic C_2 fragments of the (CH_3CO-) type.

$(-CH_2C^{*}O-)_r$ = Randomized C_2 fragments resulting from the dilution of $(-CH_2C^{*}O-)_i$ fragments by $(-CH_2CO-)$ fragments.

$C^{*}O:C^{*}OOH$ = Ratio of isotope in the carbonyl moiety (or carbon) to that in the carboxyl moiety (or carbon) of the total AAA formed.

R = $C^{*}O:C^{*}OOH$ ratio for a non-terminally-labelled fatty acid.

R_t = $C^{*}O:C^{*}OOH$ ratio for a terminally-labelled fatty acid.

Molar s.a. = Molar specific activity; arbitrary isotope concentration per mole of fatty acid or C_2 fragment.

a = Molar s.a. of $(-CH_2C^{*}O-)_r$ fragments from a non-terminally-labelled fatty acid.

a_t = Molar s.a. of $(-CH_2C^{*}O-)_r$ fragments from a terminally-labelled fatty acid.

x = Moles of AAA (*Species A*) arising from condensation of (CH_3CO-) or $(CH_3C^{*}O-)_i$ fragments and $(-CH_2C^{*}O-)_r$ fragments.

y = Moles of AAA (*Species B*) arising from condensation of $(-CH_2C^{*}O-)_r$ fragments.

$(\text{s.a. CO})_t$ = Molar s.a. of carbonyl moiety (or carbon) of total AAA formed from a terminally-labelled fatty acid.

$(\text{s.a. AAA})_t$ = Molar s.a. of total AAA formed from a terminally-labelled fatty acid.

c = Number of carbons per molecule of fatty acid.

f = Fractional conversion, $(CH_3CO-) \longrightarrow (-CH_2CO-)$ or $(CH_3C^{*}O-)_i \longrightarrow (-CH_2C^{*}O-)_i$.

II. THE GENERAL CASE

1. *Non-terminally-labelled fatty acids*

Consider a fatty acid of c carbons where c = 4, 6, 8, 10, or 12. Let us assume the given fatty acid to contain an isotope in any odd carbon except the terminal two carbons distal to the carboxyl group. For convenience, let this fatty acid have a molar isotopic concentration of unity in any arbitrary units, *i.e.*, molar s.a. = 1. After the conversion of one mole of the fatty acid into C_2 fragments, the terminal type fragments, (CH_3CO-), will have a molar s.a. = 0, while the isotopic fragments, $(-CH_2C^{*}O-)_i$, will have a molar s.a. = 1. If we now consider that isotopic fragments randomize with non-isotopic fragments from the fatty acid as well as from other sources, randomized fragments, $(-CH_2C^{*}O-)_r$, will be formed which, in general, have a molar s.a. = $a < 1$. Condensation of (CH_3CO-) fragments with $(-CH_2C^{*}O-)_r$ fragments yields, let us say, x moles of *Species (A)* of AAA, while condensation of $(-CH_2C^{*}O-)_r$ fragments (not necessarily all) yields y moles of AAA of *Species (B)*, as indicated below.

$$x \text{ moles} \quad \underbrace{CH_3CO}_{\text{s.a.} = 0} \quad \underbrace{CH_2C^{*}OOH}_{\text{s.a.} = a} \qquad (\textit{Species A})$$

$$y \text{ moles} \quad \underbrace{CH_3C^*O}_{\text{s.a.} = a} \quad \underbrace{CH_2C^*OOH}_{\text{s.a.} = a} \qquad (\textit{Species } B)$$

Species A will be referred to as being formed by non-random condensation, and *Species B* by random condensation.

The C*O:C*OOH ratio, designated here as R, is given by

$$R = C^*O{:}C^*OOH = \frac{ya}{xa + ya} = \frac{y}{x + y} \qquad \text{Eq. (1)}$$

and upon rearrangement:

$$1 - R = \frac{x}{x + y} \qquad \text{Eq. (1a)}$$

But $y/(x + y)$ represents the fraction of the AAA formed by random condensation, and $x/(x + y)$ represents the fraction formed by non-random condensation. For non-terminally-labelled fatty acids, the C*O:C*OOH ratio assumes added significance. *The C*O:C*OOH ratio, R, represents the fraction of the total AAA formed which results from random condensation, and 1—R represents the fraction resulting from non-random condensation.*

Notice should be taken that the interpretation given above for the quantities R and $1—R$ places no restriction upon whether or not 1. complete conversion of the fatty acid to C_2 fragments occurs[†]; 2. ($-CH_2CO-$) fragments may be utilized for other processes than AAA formation, or 3. C_2 fragments from sources other than the fatty acid are incorporated into AAA[††]. The freedom from these limitations thus constitutes the General Case. It is clear that the production of non-isotopic, endogenous (CH_3CO-) type fragments does not influence the interpretation that R represents the fraction of AAA resulting from random condensation. However, since in Section II-2 equations are developed for AAA formation from terminally-labelled fatty acids, we shall assume that only ($-CH_2CO-$) type fragments are produced endogenously. If the number of endogenous (CH_3CO-) type fragments which randomize with those from the fatty acid is small compared with those from the fatty acid, the above assumption regarding endogenous C_2 fragments is justified. (In this connection see also note page 40.)

Several factors may affect the relative proportions of *Species A* and *B* of AAA, and the interplay of these would then determine the ratio, R. These factors are:

1. fractional conversion of $(CH_3CO-) \longrightarrow (-CH_2CO-)$;
2. nature of interaction of C_2 fragments from other sources with those from the fatty acid;
3. extent of entrance of C_2 fragments into the tricarboxylic acid cycle or utilization for other processes;
4. extent of conversion of C_2 fragments to AAA.

The quantity R tends to increase 1. if ($-CH_2CO-$) fragments from other sources interact with those from the fatty acid, or 2. as the chain length of the fatty acid increases. In both cases the quantity y increases, effecting a less asymmetric distribution of isotope between the carbonyl and carboxyl moieties of the total AAA. Thus in Eq. (1),

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[†] For example, instead of starting with one mole of the fatty acid we could specify k moles. Then kx and ky moles of species A and B, respectively, would have been formed, and the factor k would cancel out in Eqs. (1) and (1a).

[††] CRANDALL *et al.*[5,6] have shown that acetate and pyruvate (by decarboxylation of the carboxyl carbon) give rise to a ($-CH_2CO-$) type of C_2 fragment.

with $x \neq 0$, y may vary between zero and a number larger than any assigned number (infinity). The limits of R are obviously

$$0 \leq R \leq 1.$$

In this connection GEYER, CUNNINGHAM AND PENDERGAST[7], working with liver slices, observed that this ratio for short-chain fatty acids labelled in the carboxyl carbon does indeed increase toward unity with increasing chain length[†]. In no case with non-terminally-labelled short-chain fatty acids has the $C^*O:C^*OOH$ ratio, R, been found to be significantly greater than unity.

The upper limit for R, namely, unity, was observed by WEINHOUSE, MEDES AND FLOYD[1] and by GOLDMAN *et al.*[10] in experiments in which octanoate-1-^{13}C or -1-^{14}C was incubated with fasted rat liver slices. According to the theory presented here, the AAA formed in those two cases consists almost entirely of *Species B*, the amount of *Species A* being negligible in comparison. This could result if a large number of endogenous ($-CH_2CO-$) fragments mixed with those from the fatty acid, as has been suggested previously[5][††].

It is not surprising that different ratios have been obtained with the same labelled fatty acid in different systems. Liver slices, which accumulate considerable AAA during the oxidation of fatty acids, tend to yield AAA with high values for R, whereas the ratios obtained with the same labelled fatty acid in kidney slices, which accumulate minimal amounts of acetoacetate, are low. Thus GEYER, CUNNINGHAM AND PENDERGAST[7] found that the $C^*O:C^*OOH$ ratio, R, of AAA from octanoate-1-^{14}C was about 0.7 in liver slices, but was as low as 0.21 in kidney slices. Since R represents the fraction of AAA arising from random condensation of ($-CH_2CO-$) type fragments, only 21% of the AAA formed in kidney slices results from *random* condensation, while 79% results from *non-random* condensation. If the substrate (octanoate) were the only source of (CH_3CO-) type fragments, the conclusion reached would be that at least 79 molar per cent of the carbonyl moiety of AAA formed by kidney slices was derived from the terminal two carbons of octanoate. Since the carbonyl moiety contains one-half the total atoms of carbon of AAA, *at least 39% of the total carbon of the AAA produced in the kidney slices was derived from the terminal two carbons of octanoate in kidney.* If the conversion (CH_3CO-) $\longrightarrow$ ($-CH_2CO-$) proceeds to any extent whatsoever, the percentage is even higher since ($-CH_2CO-$) fragments from the terminal two carbons can be in-

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[†] GEYER *et al.* found that the ratios for odd-carbon fatty acids also increased with increasing chain length. D. D. CHAPMAN, in our laboratories, has found that the terminal three-carbon fragment from an odd-carbon fatty acid (pentadecanoic) apparently is not converted appreciably to a (CH_3CO-) type fragment (unpublished observations). It is conceivable, therefore, that a small amount of endogenous (CH_3CO-) type fragment is responsible for the effect with odd-carbon fatty acids. Carboxyl-labelled acetate, in the washed liver homogenate system where little or no endogenous production of C_2 fragments occurs, gives rise to acetoacetate with equal concentrations of isotope in the carbonyl and carboxyl carbons[5]. However, when carboxyl-labelled acetate is incubated with nonisotopic octanoate in this washed liver homogenate, the isotope is found in higher concentration in the carboxyl carbon than in the carbonyl carbon[5]. In *liver slices*, where endogenous production of C_2 fragments normally occurs, carboxyl-labelled acetate also gives rise to acetoacetate with a similar asymmetry of incorporation of the isotope[13]. These observations indicate that a ($-CH_2CO-$) type of fragment is formed from acetate which, under the influence of (CH_3CO-) type fragments, is diverted preferentially to the carboxyl moiety of acetoacetate. Similarly, if C_2 fragments of odd-carbon fatty acids arising from carbons other than the terminal three carbons give rise to ($-CH_2CO-$) type fragments, they would then be directed preferentially to the carboxyl moiety of acetoacetate *in liver slices.*

[††] Note that this is not due to the extent of dilution of the ($-CH_2C^*O-$)$_i$ fragment *per se*, as R is independent of a.

corporated also into the carbonyl moiety of *Species B* and into the carboxyl moiety of both *Species A* and *B* of AAA.

The above considerations allow us to draw two conclusions: 1. since random condensation of $(\text{-CH}_2\text{C}^*\text{O-})_r$ fragments forming *Species B* is less in the case of kidney, the incorporation of isotope into AAA should be less in kidney than in liver, and 2. since more $(\text{-CH}_2\text{C}^*\text{O-})_r$ fragments must then be available for other processes in kidney, owing to their low incorporation into AAA, the isotope percentage incorporated into CO_2 should be higher for kidney than for liver.

Results of two typical experiments with kidney and liver slices, carried out by GEYER, CUNNINGHAM AND PENDERGAST[7], are given in Table I. These results are qualitatively in agreement with the conclusions drawn above.

TABLE I

OXIDATION OF OCTANOATE-1-^{14}C BY LIVER AND KIDNEY SLICES*

(See text, Section II)

*Tissue***	*^{14}C-c.p.m. in octanoate-1-^{14}C****	*^{14}C-c.p.m. in CO_2*	*^{14}C-c.p.m. in AAA*	$R = \frac{C^*O}{C^*OOH}$
Liver	61,300	4,370	14,980	0.60
Kidney	64,200	21,700	4,150	0.23

* Reported by GEYER, CUNNINGHAM AND PENDERGAST[7], Expt. No. 164.
** Approximately equal dry weights of tissue: liver 0.2428 g; kidney, 0.2321 g.
*** Substrate concentration, 0.001 *M*.

2. *Terminally-labelled fatty acids*

Let us now consider a fatty acid which contains isotope in the penultimate (ω—1) carbon, for example, $CH_3C^*H_2(CH_2)_{c-3}COOH$. Terminal type fragments $(CH_3C^*O\text{-})_i$ will now have a molar s.a. = 1, while the isotopic $(\text{-CH}_2\text{C}^*\text{O-})_i$ fragments derived from the conversion $(CH_3C^*O\text{-})_i \rightarrow (\text{-CH}_2\text{C}^*\text{O-})_i$ may randomize with any non-isotopic $(\text{-CH}_2\text{CO-})$ type fragments to form randomized fragments $(\text{-CH}_2\text{C}^*\text{O-})_r$ of molar s.a. = a_t. The two species of AAA may therefore be represented as

$$x \text{ moles} \quad \underbrace{CH_3C^*O}_{\text{s.a.} = 1} \quad \underbrace{CH_2C^*OOH}_{\text{s.a.} = a_t} \qquad \textit{Species A}$$

$$y \text{ moles} \quad \underbrace{CH_3C^*O}_{\text{s.a.} = a_t} \quad \underbrace{CH_2C^*OOH}_{\text{s.a.} = a_t} \qquad \textit{Species B}$$

The C*O:C*OOH ratio is given as

$$R_t = \frac{C^*O}{C^*OOH} = \frac{x + ya_t}{xa_t + ya_t} \qquad \text{Eq. (2)}$$

Eq. (2) may be rearranged to

$$R_t = \frac{y}{x + y} + \frac{x}{a_t\,(x + y)} \qquad \text{Eq. (2a)}$$

Eqs. (1) and (2a) lead to

$$a_t = \frac{1 - R}{R_t - R} \qquad \text{Eq. (3)}$$

Since the molar s.a. of the carboxyl moiety of both *Species A* and *B* is a_t, the molar s.a. of the total carboxyl moiety of AAA is also a_t. Eq. (3) expresses, then, the relation between the molar s.a. of the carboxyl moiety of AAA for the case of a terminally-labelled fatty acid of an even number of carbons and the two C*O:C*OOH ratios, R and R_t.

Since the C*O:C*OOH ratio can also be expressed in terms of specific activities

$$R_t = \frac{C^*O}{C^*OOH} = \frac{\text{molar s.a. carbonyl moiety}}{\text{molar s.a. carboxyl moiety}},$$

$$\text{or} \quad R_t = \frac{(\text{s.a. CO})_t}{a_t} \qquad \text{Eq. (4)}$$

Eqs. (3) and (4) lead to

$$(\text{s.a. CO})_t = \frac{1 - R}{1 - R/R_t} \qquad \text{Eq. (5)}$$

where $(\text{s.a. CO})_t$ = molar s.a. of the carbonyl moiety of AAA for the case of a terminally-labelled fatty acid.

ZABIN AND BLOCH[11] incubated butyrate-1-^{13}C, -3-^{14}C with rat liver slices and found $R = 0.428$ and $R_t = 3.49$. Substitution of these values into Eqs. (3) and (5) leads to $a_t = 0.187$ and $(\text{s.a. CO})_t = 0.652$. ZABIN AND BLOCH reported the following values:

$$\frac{\text{c.p.m. } ^{14}\text{C in COOH of AAA}}{\text{c.p.m. } ^{14}\text{C in C-3 of butyrate}} = 0.188$$

$$\frac{\text{c.p.m. } ^{14}\text{C in CO of AAA}}{\text{c.p.m. } ^{14}\text{C in C-3 of butyrate}} = 0.656$$

The theoretical values for a_t and $(\text{s.a. CO})_t$ which were calculated using only the experimentally determined ratios R and R_t agree *numerically* with the ratios of

$$\frac{\text{c.p.m. } ^{14}\text{C in COOH or CO of AAA}}{\text{c.p.m. } ^{14}\text{C in C-3 of butyrate}}.$$

Such numerical agreement must be fortuitous, however. In general, ^{14}C data alone give no information as to specific activities, and a_t and $(\text{s.a. CO})_t$ are defined as molar specific activities. The ratios given above involving c.p.m. ^{14}C become identical with a_t and $(\text{s.a. CO})_t$ when AAA arises from both butyrate and endogenous C_2 fragments only 1. if the butyrate is completely utilized, and 2. if for each mole of butyrate that disappears, one mole of AAA is formed. 1. is of incidental importance only, but 2. permits calculations to be made a. for the extent of the conversion $(CH_3{}^{14}CO\text{–})_i \longrightarrow (\text{–}CH_2{}^{14}CO\text{–})_i$, b. for the value of the molar s.a., a, of the $(\text{–}CH_2C^*O\text{–})_r$ fragments with respect to ^{13}C, and c. for the direct verification from the data of ZABIN AND BLOCH[11] to the effect that endogenous C_2 fragments are incorporated into AAA with essentially the same asymmetry as the carboxyl C_2 fragment, $(\text{–}CH_2{}^{13}CO\text{–})_i$, of butyrate. These calculations are as follows:

a. Recalling that R is numerically equal to the fraction of AAA arising from the condensation of $(\text{–}CH_2C^*O\text{–})_r$ fragments, one mole of AAA formed during the utilization of one mole of butyrate-3-^{14}C of molar s.a. = 1 may be represented as

$$1 - R = x = 0.572 \text{ moles} \quad \underbrace{CH_3{}^{14}CO}_{\text{s.a.} = 1} \quad \underbrace{CH_2{}^{14}COOH}_{\text{s.a.} = 0.187} \qquad (\textit{Species A})$$

$$R = y = 0.428 \text{ moles} \qquad \underbrace{CH_3{}^{14}CO}_{\text{s.a.} = 0.187} \qquad \underbrace{CH_2{}^{14}COOH}_{\text{s.a.} = 0.187} \qquad (\textit{Species B})$$

Since, during the utilization of one mole of butyrate, 0.572 moles of *Species A* of AAA are formed, one mole of $(CH_3{}^{14}CO\text{–})_i$ fragments must have been converted to (1.000 — 0.572) = 0.428 moles of $(\text{–}CH_2{}^{14}CO\text{–})_i$ fragments. Thus, the fractional conversion, f, for $(CH_3CO\text{–}) \longrightarrow (\text{–}CH_2CO\text{–})$ is equal to 0.428 for butyrate in liver slices. It will be shown in Section III that this figure is comparable to that calculated for other short-chain fatty acids in washed rat liver homogenates.

b. Since all fragments of the $(\text{–}CH_2CO\text{–})$ type which interact with one another are assumed to randomize, the molar s.a. of $(\text{–}CH_2C^*O\text{–})_r$ fragments with respect to ^{14}C or ^{13}C must be proportional to the relative number of $(\text{–}CH_2C^*O\text{–})_i$ fragments of each kind (^{14}C- or ^{13}C-labelled) which undergo randomization. Thus, for each mole of butyrate utilized, 0.428 moles of $(\text{–}CH_2{}^{14}CO\text{–})_i$ fragments and 1.000 moles of $(\text{–}CH_2{}^{13}CO\text{–})_i$ fragments are formed. The molar s.a. of $(\text{–}CH_2C^*O\text{–})_r$ fragments with respect to ^{14}C was shown to be equal to 0.187; therefore the molar s.a., a, of $(\text{–}CH_2C^*O\text{–})_r$ fragments with respect to ^{13}C must be 0.187/0.428 = 0.437. We may now represent the AAA formed during the utilization of one mole of butyrate-1-^{13}C of molar s.a. = 1 as

$$1 - R = x = 0.572 \text{ moles} \qquad \underbrace{CH_3CO}_{\text{s.a.} = 0} \qquad \underbrace{CH_2{}^{13}COOH}_{\text{s.a.} = 0.437}$$

$$R = y = 0.428 \text{ moles} \qquad \underbrace{CH_3{}^{13}C}_{\text{s.a.} = 0.437} \qquad \underbrace{CH_2{}^{13}COOH}_{\text{s.a.} = 0.437}$$

Hence, (0.428) (0.437) = 0.187 and (0.572 + 0.428) (0.437) = 0.437 moles of $(\text{–}CH_2{}^{13}CO\text{–})_i$ fragments reside in the carbonyl and carboxyl moieties, respectively, of the AAA. Since only one mole of AAA is formed per mole of butyrate utilized, the molar s.a.'s of the carbonyl and carboxyl moieties with respect to ^{13}C are 0.187 and 0.437, respectively. The corresponding molar s.a.'s reported by ZABIN AND BLOCH† were 0.196 and 0.458.

c. The sum of the moles of ^{14}C- and ^{13}C-labelled C_2 fragments and endogenous C_2 fragments in the two moieties of AAA must equal 1.000. The moles of the isotopic fragments in the two moieties are numerically equal to the respective molar s.a.'s of these moieties. Hence, the number of moles of endogenous C_2 fragments in either moiety is obtained by subtracting the sum of the molar s.a.'s for ^{14}C- or ^{13}C-labelled C_2 fragments for that moiety from unity, *i.e.*, from the number of moles of AAA formed when one mole of butyrate is utilized. This C_2 fragment balance is indicated in Table II. It is clear from Table II that endogenous C_2 fragments (^{12}C) are incorporated into AAA with essentially the same asymmetry as the carboxyl C_2 fragments (^{13}C) of butyrate.

The molar s.a. of AAA, $(\text{s.a. AAA})_t$, is given by the sum of the molar s.a.'s of the carbonyl and carboxyl moieties. Thus addition of Eq. (3) to Eq. (5) gives

$$(\text{s.a. AAA})_t = (\text{s.a. CO})_t + a_t = \frac{(1 - R)\,(1 + R_t)}{R_t - R} \qquad \text{Eq. (5a)}$$

where $(\text{s.a. AAA})_t$ is the molar s.a. of the AAA for the case of a terminally-labelled fatty acid. Except where small amounts of AAA are formed from fatty acids, as was

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† Reported as $\dfrac{\text{atom per cent excess } {}^{13}C \text{ in CO or COOH of AAA}}{\text{atom per cent excess } {}^{13}C \text{ in C-1 of butyrate}}$.

TABLE II

INCORPORATION OF ^{13}C- AND ^{14}C-LABELLED C_2 FRAGMENTS FROM BUTYRATE-1-^{13}C, -3-^{14}C INTO ACETOACETATE MOIETIES IN THE PRESENCE OF LIVER SLICES*

*Theoretical***				*Observed****			
Isotopic C_2 fragment	*Moles of isotopic C_2 fragments in AAA moieties per mole of butyrate utilized*		$\frac{CO}{COOH}$	*Isotopic C_2 fragment*	*Moles of isotopic C_2 fragments in AAA moieties per mole of butyrate utilized*		$\frac{CO}{COOH}$
	CO	*COOH*			*CO*	*COOH*	
^{14}C	0.652	0.187	$R_t = 3.49$	^{14}C	0.656	0.188	$R_t = 3.49$
^{13}C	0.187	0.437	$R = 0.428$	^{13}C	0.196	0.458	$R = 0.428$
^{12}C (endogenous)	0.161	0.376	0.428	^{12}C (endogenous)	0.148	0.354	0.418

* Assuming that one mole of AAA is formed during the utilization of one mole of butyrate, justification of which is given in the text.

** Calculated on the basis that $R_t = 3.49$ and $R = 0.428$ as observed.

*** Taken from the data of ZABIN AND BLOCH[11].

discussed in connection with kidney, the quantity (s.a. AAA)$_t$ is generally less than unity. In this case the limits of R_t are readily obtained. For if (s.a. AAA)$_t \leq 1$, we have from Eq. (5a)

$$\frac{(1 - R)\ (1 + R_t)}{R_t - R} \leq 1$$

$$\text{or } R_t\text{R} \geq 1$$

Recalling that $0 \leq R \leq 1$, the limits of R_t may then be given as

$$1 \leq R_t \leq \text{p} \quad \text{if (s.a. AAA)}_t \leq 1$$

where p is greater than any assigned number.

It is interesting to note how (s.a. AAA)$_t$ may be greater than unity although the molar s.a. of a fatty acid is unity. This can result as follows: Suppose that one mole of butyrate-3-^{14}C is converted to one mole each of $(CH_3C^*O-)_i$ and $(-CH_2CO-)$ fragments, and that 0.5 mole of the former is converted to $(-CH_2C^*O-)_i$ fragments. This would result in 0.5 moles of $(CH_3C^*O-)_i$ with molar s.a. = 1.0, and 1.5 moles of $(-CH_2C^*O-)_r$ with molar s.a. $= \frac{0.5}{1.5} = 0.333$. If, now, 1.0 mole of $(-CH_2C^*O-)_r$ condenses with oxalacetate and is oxidized to CO_2 or proceeds along synthetic pathways other than AAA formation, the remaining C_2 fragments may condense to give 0.5 mole of AAA. The carbonyl moiety will have a molar s.a. = 1, and the carboxyl moiety a molar s.a. = 0.333. The (s.a. AAA)$_t$ would then just be $1 + 0.333 = 1.333$. Note that in this case, $R_t = 3.0$. If carboxyl-butyrate had been used, R would be zero, *i.e.*, the isotope would be found only in the carboxyl carbon. The low values observed for R with a carboxyl-labelled fatty acid in kidney slices indicate that a process similar to the example given above is occurring in the kidney slice. *That is to say, AAA from even fatty acids in kidney arises in large measure from the terminal two carbons.*

Inspection of Eqs. (4) and (5a) reveals that the molar s.a.'s of the carbonyl and carboxyl moieties of AAA for a terminally-labelled fatty acid are exceptionally useful quantities. Their sum and quotient give (s.a. AAA)$_t$ and R_t, respectively, and hence R may be determined from Eq. (5a) after substitution and rearrangement.

It should be emphasized again that the relationships so far derived are general, in that C_2 fragments from the fatty acid do not necessarily have to be completely converted into AAA, but may be available for other synthetic processes, and that ($-CH_2CO-$) type fragments from sources other than the fatty acid can simultaneously be incorporated into AAA.

Unfortunately, sufficient data are, as yet, unavailable for a further verification of the analysis of the General Case. In most of these instances, where AAA formation would be amenable to the General Case, only the C^*O:C^*OOH ratio, R, for the non-terminally-labelled fatty acids has been determined. In view of the multiplicity of possible factors involved (see above), more information is needed, such as the knowledge of the quantities R_t, a_t, or (s.a. AAA)$_t$, to verify quantitatively the mechanism predicted by the analysis for the General Case. However, the washed liver homogenate system, as used by CRANDALL *et al.*[5,6], presents fewer variables, and we have found that such a system represents a "restricted" case to which the equations derived for the General Case apply.

III. A RESTRICTED CASE: WASHED LIVER HOMOGENATES

The washed liver homogenate system presents a restricted and simple case because the C_2 fragments from the fatty acid may be incorporated nearly quantitatively into AAA[5,6,16].

Several important relationships may be obtained for this restricted case. The number of moles of AAA formed from one mole of the fatty acid is given by $x + y$ (by definition). If c = number of carbons per molecule of the fatty acid:

$$c/4 = x + y \qquad \text{Eq. (6)}$$

Eqs. (1) and (6) yield

$$y = (c/4)R \qquad \text{Eq. (7)}$$

$$x = (c/4)\,(1 - R) \qquad \text{Eq. (8)}$$

Since we may assume that the C_2 fragments from one mole of the fatty acid appear quantitatively in AAA, and since the molar s.a. of the fatty acid was taken as unity, the total isotopic content of AAA from one mole of fatty acid must be unity.

Therefore:

$$xa + 2\,ya = 1 \qquad \text{Eq. (9)}^{\dagger}$$

or

$$a = \frac{1}{x + 2y} \qquad \text{Eq. (9a)}^{\dagger}$$

CRANDALL AND GURIN[5], and CRANDALL, BRADY AND GURIN[6] have found that octanoate-1-^{13}C or -1-^{14}C yields a C^*O:C^*OOH ratio, R, of about 0.7[††]. Using a value of 0.70 for R, and $c = 8$, Eqs. (7), (8), and (9) lead to

$$x = 0.60$$
$$y = 1.40$$
$$a = 0.29$$

In seven experiments[5], the average of observed values for the molar s.a. of the

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[†] Eqs. (9) and (9a) are obtained in the same form if, in more general terms, k moles of the fatty acid are considered.

[††] Actually, values of 0.74 and 0.75 were observed in experiments with octanoate-1-^{14}C, and an average value of 0.66 with octanoate-1-^{13}C (refs. 6 and 5, respectively).

carboxyl carbon, which is equivalent to the quantity a, was 0.26. Although the calculated value, $a = 0.29$, appears to be in good agreement, the quantity a is relatively insensitive to changes in R, and hence to changes in x and y. The quantity, a, can only have values between $a = 0.250$ and $a = 0.333$. This results because a maximum of 3, or a minimum of 2, non-isotopic ($-CH_2CO-$) fragments may randomize with each isotopic $(-CH_2C^*O-)_i$ fragment when using octanoate, depending upon the degree of the conversion (CH_3CO-) $\longrightarrow$ ($-CH_2CO-$). The resulting randomized fragments, $(-CH_2C^*O-)_r$, will then have a molar s.a. within the narrow range of 1/3 to 1/4. A slight contamination of CO_2 (as well as of acetone) in the determination of the s.a. of the carboxyl carbon has been indicated by CRANDALL *et al.*[5,6]. If this contaminating CO_2 has a lower s.a. than that of the CO_2 derived from the carboxyl carbon of AAA, it would be expected that the observed average value of a (0.26) would be lower than that predicted according to theory (0.29). The observed $C^*O:C^*OOH$ ratio would also be somewhat in error since the *ratio* of specific activities of the carbonyl and carboxyl carbons of AAA was used to determine this ratio. However, a small change in R effects an even smaller change, percentagewise, in the quantity a. We conclude that the observed value of a agrees, within experimental error, with that calculated from the observed value for R in the experiments of CRANDALL *et al.* Equations (7), (8), and (9) thus provide a useful check on experimental procedures in the washed liver homogenate system under discussion.

If $x = 0.60$, the conversion (CH_3CO-) $\longrightarrow$ ($-CH_2CO-$) must be $(1.00 - 0.60)\ 100 = 40\%$, or one mole of terminal two carbons gives rise to 0.40 mole of ($-CH_2CO-$) fragments identical with those derived from the rest of the molecule. Thus, the fractional conversion is given as $f = 0.4$ if $R = 0.70$. If $R = 0.75$, the following values are obtained: $x = 0.50$, $y = 1.50$, $a = 0.285$, $f = 0.5$. It will be recalled that f was calculated to be equal to 0.428 for butyrate in liver slices (Section II–2).

The usefulness of the above derived equations can be exemplified by verifying the $C^*O:C^*OOH$ ratio, R, for another fatty acid, hexanoate-1-^{13}C. If it be assumed that the fractional conversion (CH_3CO-) $\longrightarrow$ ($-CH_2CO-$) is identical with that of octanoate for $R = 0.70$, then $f = 0.4$ and $x = 0.60$, and the observed ratio is given by Eq. (1a) rearranged as follows:

$$R = 1 - \frac{x}{x+y} = 1 - \frac{0.60}{x+y} \qquad \text{Eq. (1b)}$$

But $x + y = c/4$, and $c = 6$ for hexanoate; therefore $R = 1 - \frac{0.60}{1.5} = 0.60$. The ratios observed by CRANDALL AND GURIN[5] were 0.58 and 0.56 for two experiments with hexanoate. *The theoretical confirmation of the ratio observed with hexanoate-1-^{13}C illustrates in a striking fashion the applicability of the concept of two types of C_2 fragments.*

For the special case under consideration, another important relationship may be derived. Since the molar s.a. of the fatty acid was taken as unity, the total isotopic content of the AAA from *one mole* of the fatty acid must be unity. The moles of AAA formed from one mole of fatty acid of c carbons is equal to $c/4$; hence the molar s.a. of the AAA must be $\frac{1}{(c/4)} = 4/c$. Substitution of $4/c$ for $(\text{s.a. AAA})_t$ in Eq. (5a) derived for the General Case gives, for the restricted case under consideration,

$$4/c = \frac{(1 - R)\,(1 + R_t)}{R_t - R} \qquad \text{Eq. (10)}$$

In their liver homogenate system, CRANDALL *et al.*[5,6] found $R = 0.7$ with $c = 8$, *i.e.*, with carboxyl-labelled octanoate. Substitution of these values into Eq. (10) gives $R_t = 3.3$. The values found ranged from 2.9 to 3.6 for three observations[6]. The reverse calculation was made in another manner by these investigators; using the average of the observed values, $R_t = 3.3$, it was shown that the expected ratio, R, should be 0.7.

The preceding theoretical treatment of the restricted case may be represented graphically to illustrate its application in washed liver homogenates. To do this, Eq. (10) is solved for several R_t's (taking positive values only) by substituting values of R from $R = 0$ to $R = 1$ for $c = 4, 6, 8, \ldots$ etc. By plotting R_t vs. R for $c = 4, 6, 8 \ldots$ etc., a family of curves is obtained (see Figs. 1 and 2). If, now, the fractional conversion, f, of (CH_3CO–) type fragments to (–CH_2CO–) type fragments is located on each curve of $c = 4, 6, 8, \ldots$ for the corresponding value of R, straight lines, representing the fractional conversion of terminal fragments, may be drawn which intersect the family of curves. The fractional conversion, f, is located on each curve as follows: Since x represents the moles of AAA due to unconverted (CH_3CO–) fragments from one mole of a fatty acid, the quantity $1 - x$ is just the fractional conversion, *i.e.*, f. Thus $f = 1 - x$. The corresponding values of R for each c when $f = 0.0, 0.1, 0.2, \ldots 1.0$ may then be calculated from Eq. (8) with the substitution, $x = 1 - f$.

It will be noted that the maximum value of R is unity for all curves, and this maximum value obtains when $f = 1.0$, or when c increases without bounds. A minimum value of R is approached for each fatty acid when $f = 0$. The minimum values of R for the various fatty acids, and values for x and y of *Species A* and *B* of AAA are summarized in Table III. The minimum values of R for each fatty acid are represented graphically by the $R = k$ which each curve approaches asymptotically as the fractional conversion f approaches zero. As f approaches 0, R_t increases without bounds for each fatty acid. The lower limit of R_t is unity.

The applicability of these graphs is shown in the examples presented below:

Example 1. Given any two of the values, R, c, R_t, or f, the other two are readily obtained. Thus if $R = 0.7$ for octanoate is located on curve $c = 8$, values for $R_t = 3.25$

TABLE III

MINIMUM VALUES OF R FOR THE RESTRICTED CASE

($f = 0$) (See text, Section III)

Fatty acid	*c = no. of carbons in fatty acid*	x	y	R
Butyrate	4	1.00	0	0
Hexanoate	6	1.00	0.50	0.33
Octanoate	8	1.00	1.00	0.50
Decanoate	10	1.00	1.50	0.60
Dodecanoate	12	1.00	2.00	0.67
—	(14)*	1.00	2.50	0.71
—	(16)*	1.00	3.00	0.75
—	(18)*	1.00	3.50	0.78
—	(20)*	1.00	4.00	0.80

* These values merely specify the carbons of a fatty acid which contains C_2 fragments equivalent in number to those from a lower chain fatty acid plus additional (–CH_2CO–) type fragments. Thus $c = 16$ indicates one (CH_3CO–) type fragment and 7 (–CH_2CO–) type fragments. This is equivalent to that produced when pyruvate is added to octanoate so as to double the number of C_2 fragments arising from octanoate (see footnote †† page 39 and text).

and for $f = 0.4$ may be read from the ordinate and diagonal lines, respectively. If for hexanoate, $f = 0.4$, as was the case with octanoate, R for hexanoate is read as 0.60, and R_t as 3.00.

Example 2. If C_2 fragments of the ($-CH_2CO-$) type from sources other than a given fatty acid (for example, from *added* pyruvate) interact with those of the fatty acid, the pattern of AAA formation would be the same as if a longer chain fatty acid than the given fatty acid were employed (assuming f = constant). (See footnote to Table III). As more and more ($-CH_2CO-$) fragments interact with those from the given fatty acid, both R and R_t increase. As shown in Fig. 1, R increases linearly with R_t.

For the case where $f = 0.4$, as with octanoate-1-^{14}C ($R = 0.70$), the ratio R_t can never be greater than 4.0, *i.e.*, the point where $f = 0.4$ intersects $R = 1.0$.

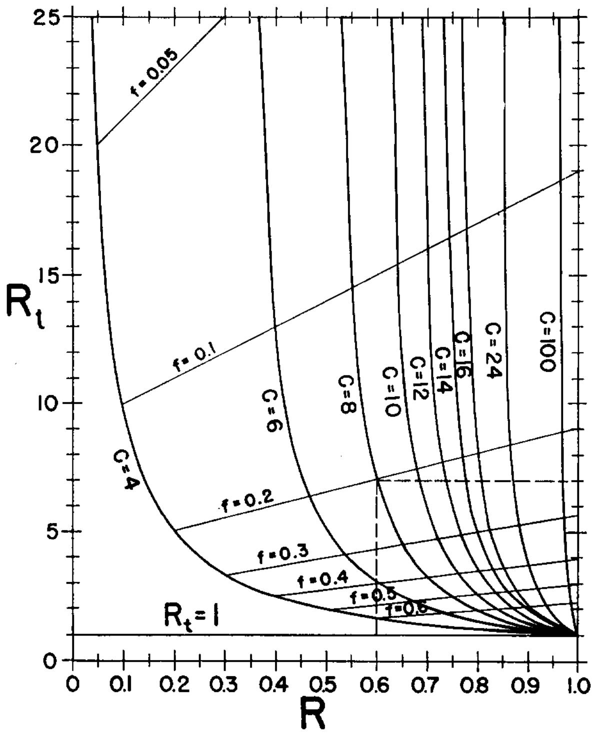

Fig. 1. Plot of R vs. R_t for short-chain fatty acids in the washed liver homogenate system. Legend: c, number of carbons in the fatty acid, or in cases above $c = 12$ in the sense discussed in the text; R_t and R, C*O:C*OOH ratios for terminally and non-terminally-labelled fatty acids, respectively; f, fractional conversion of (CH_3CO-) $\longrightarrow$ ($-CH_2CO-$). Dotted region is expanded in Fig. 2.

Example 3. CRANDALL *et al.*[5,6] observed that the ratio R for octanoate-1-C* increased from 0.7 to 0.86 as pyruvate was added (see [††] page 39), but observed no increase in the ratio R_t from the value 3.3. However, since the value for R_t could not, according to theory, be greater than 4.0, if $f = 0.4$ (see Example 2), it is not surprising that an increase was not observed. Pyruvate was added at a concentration sufficient to double the number of C_2 fragments produced when octanoate was incubated alone. Hence the ratios R and R_t will be determined by the intersection of $c = 16$ with $f = 0.4$ on the graph. Inspection reveals that, at this intersection, R has a value of 0.85, and R_t a value of 3.6. Only a slight increase in R_t results, and the experimental variations were greater than the calculated increase (see Table IV). Even if f changed with the added pyruvate, R cannot, in this case, be less than 0.75 (see Table III for $c = 16$).

CRANDALL, BRADY AND GURIN[6] have calculated that the ratio with octanoate-1-^{14}C should increase from 0.7 to 0.86 with the added pyruvate. However, this method of calculation should be regarded only as a good approximation if f remains constant, since it was assumed that the distribution of the terminal fragment in the carbonyl and carboxyl moieties of AAA was the same with or without the added pyruvate. In other words, these workers assumed that R_t did not change with added pyruvate. That the approximation was a good one is supported in two ways: 1. no significant increase in R_t was observed with added pyruvate; and 2. the slope of $f = 0.4$ is such as to indicate only a slight increase in R_t with the amount of added pyruvate.

Example 4. The C*O:C*OOH ratios obtained with octanoate-1-^{14}C and -7-^{14}C, by CRANDALL *et al.*[5,6], are explainable on a basis of $f = 0.5$ as well as $f = 0.4$. This results, as shown in Figs. 1 and 2, from the fact that the line $f = 0.4$ intersects $c = 8$ at $R = 0.70$, giving $R_t = 3.25$, and $f = 0.5$ intersects $c = 8$ at $R = 0.75$, giving $R_t = 2.50$. Since for either $f = 0.4$ or $f = 0.5$, either R or R_t falls within the range of values observed by CRANDALL *et al.*[5,6], we cannot distinguish between $f = 0.4$ or $f = 0.5$. A summary of values of R and R_t predicted by the theory for $f = 0.4$ and $f = 0.5$ is given in Table IV.

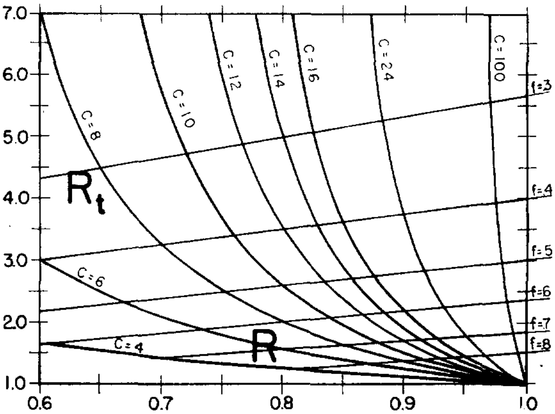

Fig. 2. Expanded portion of Fig. 1. $f = 1.0$, which is not indicated, is represented by the point (1, 1).

TABLE IV

PREDICTED VALUES FOR C*O:C*OOH RATIOS OF SHORT-CHAIN FATTY ACIDS REPRESENTING THE RESTRICTED CASE (SEE TEXT, SECTION III)

Substrate	*C*O:C*OOH theoretical values*		*C*O:C*OOH observed*
	$f = 0.4$†	$f = 0.5$†	
Butyrate-1-^{14}C	0.40	0.50	—
Butyrate-3-^{14}C	2.50	2.00	—
Hexanoate-1-^{14}C	0.60	0.67	0.56††, 0.58†† (Ref. 5)
Octanoate-1-^{14}C	0.70	0.75	0.74, 0.75 (Ref. 6), 0.66††, ††† (Ref. 5)
Octanoate-7-^{14}C	3.25	2.50	3.6, 2.9, 3.5 (Ref. 6)
Decanoate-1-^{14}C	0.76	0.80	—
Decanoate-9-^{14}C	3.40	2.60	—
Dodecanoate-1-^{14}C	0.80	0.83	—
Dodecanoate-11-^{14}C	3.50	2.65	—
Octanoate-1-^{14}C + pyruvate§	0.85	0.88	0.81, 0.82, 0.82, 0.86, 1.00 (Ref. 5)
Octanoate-7-^{14}C + pyruvate§	3.63	2.75	3.0, 3.2, 3.8 (Ref. 6)

† Equal to the fractional conversion of the terminal C_2 fragment: (CH_3CO–) → (–CH_2CO–) (see text).

†† Value(s) are probably low due to methodology (CRANDALL, BRADY AND GURIN[6], p. 847).

††† Average of 7 determinations.

§ Pyruvate added in such a quantity as to double the number of C_2 fragments arising from octanoate alone. On Graph I see intersection of $f = 0.4$ and $f = 0.5$ with $c = 16$.

IV. CONCLUSIONS

From the foregoing analyses, several generalized statements may be presented relative to the C*O:C*OOH ratios, R, and R_t.

1. The C*O:C*OOH ratio, R, for non-terminally-labelled fatty acids, may vary only between zero and unity (Section II–1).

2. The corresponding ratio, R_t, for terminally-labelled fatty acids, may vary only between unity and infinity (Section II–2).

3. R increases toward unity as the chain length of the fatty acid increases, and decreases as the chain length decreases (Section II–1).

4. R_t increases without bounds as the fractional conversion, f, decreases, and decreases toward unity as this conversion increases (Section III).

5. R decreases as more (–CH_2CO–) type fragments are utilized for processes other than acetoacetate formation, and increases toward unity as more fragments of this type interact with those from the fatty acid to form AAA (Sections II–1 and II–2).

6. R increases toward unity as the conversion, f, increases, and decreases as this conversion decreases (Section III).

7. R_t may be greater than unity and finite, although R approaches infinitesimally close to unity, but only if f is not equal to zero (Section III).

8. The product (R_tR) is greater than unity if (s.a. $AAA)_t$ is less than unity (Section II–2).

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SUMMARY

1. A theoretical interpretation of acetoacetate formation from isotopically-labelled, short-chain fatty acids of the even series is given which is based upon the concept of two types of two-carbon fragments, namely, (CH_3CO–) and (–CH_2CO–), introduced by CRANDALL *et al.* The former type results exclusively from the terminal two carbons of a fatty acid, and the latter type arises from other two-carbon units of the fatty acid as well as from the conversion of some of the fragments of the (CH_3CO–) type.

2. Two species of acetoacetate are considered, one of which results from condensation of (CH_3CO–) type fragments with fragments of the (–CH_2CO–) type, while the other arises exclusively from fragments of the latter type.

3. The $C^*O:C^*OOH$ ratio for non-terminally-labelled fatty acids with isotope in an odd carbon is shown to be numerically equal to the fraction of acetoacetate arising from the condensation of (–CH_2CO–) type fragments.

4. When small amounts of acetoacetate are formed from even fatty acids, the terminal two carbons distal to the carboxyl group are the major source of acetoacetate carbons.

5. The formation of acetoacetate in slices and washed homogenates of rat liver is consistent with the view that from 40 to 50% of the terminal two carbons of a fatty acid apparently become identical in reactivity with the two-carbon fragments derived from the rest of the molecule.

6. Two-carbon fragments from non-fatty acid (endogenous) sources are incorporated into acetoacetate with a similar, if not the same, asymmetry as non-terminal two-carbon fragments.

7. In those investigations where sufficient information is available, observed values for the $C^*O:C^*OOH$ ratio or specific activities of acetoacetate moieties agree closely with those predicted by the theory presented here.

RÉSUMÉ

1. Les auteurs donnent une interprétation théorique de la formation d'acétoacétate à partir d'acides gras à chaîne brève de la série paire marqués par des isotopes, interprétation basée sur la conception introduite par CRANDALL *et al.* de deux types de fragments à deux atomes de carbone, (CH_3CO–) et (–CH_2CO–). Le premier type résulte exclusivement des deux atomes de carbone terminaux d'un acide gras; le deuxième résulte d'autres unités à deux atomes de carbone de l'acide gras et aussi de la transformation de certains des fragments du type (CH_3CO–).

2. Les auteurs considèrent deux espèces d'acétoacétate, l'une résultant de la condensation de fragments du type (CH_3CO–) avec des fragments du type (–CH_2CO–), l'autre étant consituée uniquement de fragments du deuxième type.

3. Il est montré que le rapport $C^*O:C^*OOH$ pour des acides gras qui ne sont pas marqués dans le groupe terminal mais dans un atome de carbone impair est numériquement égal à la fraction d'acétoacétate résultant de la condensation de fragments du type (–CH_2CO–).

4. Lorsque de faibles quantités d'acétoacétate se forment à partir d'acides gras, pairs, les deux atomes de carbone les plus éloignés du groupe carboxyle forment la source principale d'atomes de carbone d'acétoacétate.

5. La formation d'acétoacétate dans les tranches et les homogénats lavés de foie de rat est en accord avec l'idée que le 40 à 50% des deux atomes de carbone terminaux d'un acide gras acquiert apparemment la même réactivité que les fragments à deux atomes de carbone provenant du reste de la molécule.

6. Des fragments à deux atomes de carbone provenant d'acides endogènes autres que les acides gras sont incorporés dans l'acétoacétate avec une asymétrie semblable sinon identique à celle des fragments à 2 C non terminaux.

7. La où des données suffisantes sont à disposition, les valeurs observées pour le rapport $C^*O:C^*OOH$ ou activité spécifique de l'acétoacétate, s'accordent bien avec celles prévues par la théorie proposée dans ce mémoire.

ZUSAMMENFASSUNG

1. Es wird eine theoretische Darstellung der Acetylacetatbildung aus mit Isotopen gekennzeichneten kurzkettigen Fettsäuren mit gerader Kohlenstoffanzahl gegeben, die auf der Annahme von zweierlei Typen von Zweikohlenstoffbruchstücken, nämlich der von CRANDALL und Mitarbeitern eingeführten (CH_3CO–)- und (–CH_2CO–)-Bruchstücke, aufgebaut ist. Die erste Type stammt ausschliesslich aus den zwei endständigen Kohlenstoffatomen einer Fettsäure; die zweite Type kann aus anderen Zweikohlenstoffatomeinheiten einer Fettsäure ebenso entstehen wie durch Umwandlung einiger Bruchstücke des (CH_3CO–) Typs.

2. Es werden zwei Acetylacetatarten in Betracht gezogen: Die eine entsteht bei der Kondensation von (CH_3CO–) Bruchstücken mit Bruchstücken des (–CH_2CO–) Typs, während die andere ausschliesslich aus Bruchstücken des letzteren Typs entsteht.

3. Das $C^*O:C^*OOH$ Verhältnis für nicht endständig mit einem Isotop in einem ungeradzahligen Kohlenstoffatom gekennzeichnete Fettsäuren ist — wie gezeigt wird — zahlenmässig gleich dem bei der Kondensation aus (–CH_2CO–) Bruchstücken entstehenden Bruchteil Acetylacetat.

4. Wenn geringe Mengen Acetylacetat aus geradzahligen Fettsäuren gebildet werden, so sind die zwei am weitesten von der Carboxylgruppe entfernten endständigen Kohlenstoffatome die Hauptquellen der Acetylacetatkohlenstoffatome.

5. Die Bildung von Acetylacetat in Schnitten und gewaschenen Homogenaten von Rattenleber stimmt mit der Ansicht überein, dass 40–50% der zwei endständigen Kohlenstoffatome einer Fettsäure in der Reaktivität identisch werden mit den Zweikohlenstoffbruchstücken, die von dem Rest des Moleküls stammen.

6. Zweikohlenstoffbruchstücke von (endogen gebildeten) Nichtfettsäuren werden in Acetylacetat mit einer ähnlichen wenn nicht gleichen Unregelmässigkeit eingebaut wie die nicht endständigen Zweikohlenstoffbruchstücke.

7. Bei den Untersuchungen, in denen genügend Einzelheiten berichtet werden, stimmen die beobachteten Werte für das $C^*O:C^*OOH$-Verhältnis oder für die spezifischen Aktivitäten der Acetylacetathälften gut überein mit denen, die mit Hilfe der hier dargelegten Theorie vorhergesagt wurden.

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