THE ANATOMY OF TRANSCARBOXYLASE AND THE ROLE OF ITS SUBUNITS*

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EVENTS LEADING TO THE STUDY OF TRANSCARBOXYLASE

My scientific career began 46 years ago in a study of the propionic acid bacteria at Iowa State College, now Iowa State University. I used radioactive and stable isotopes to study the fermentation. Then, I branched into the study of metabolism of the rats and even cows, using isotopes.[1] Thinking back, I find it interesting that I made no serious effort to study metabolism at the enzymatic level for about 30 years. Although Dr. Merton Utter and I, at the University of Minnesota in the early 1940s, in an attempt to determine if glycolysis is altered during poliomyelitis, prepared crude extracts of the brains of cotton rats, which had been infected with polio, this didn't involve purification of the enzymes. Incidentally, we found no effect.[2]

It wasn't that I had little interest in enzymes; I read and admired the studies by Warburg, Meyerhof, Lipmann, Carl and Gerti Cori, Ochoa, Kornberg, and many others. However, my major interest was to prove with isotopes that what was predicted on the basis of studies with enzymes really occurred in intact animals and bacteria. The physiologists in those days were skeptical whether studies made with chopped-up tissue and enzymes had much to do with what went on in the intact animal, and some were quite surprised that it did.

What really enticed me into the field of enzymology was the propionic acid fermentation. The isotope patterns in the products obtained from ^{14}C-labeled glucose in this fermentation are most peculiar and unlike any other fermentation. For example, no matter which of the six carbons of glucose is labeled, the isotope appears in each and every position of the propionic acid.[3] It became clear that we must delve into the details of the individual reactions if we wished to understand how these isotope patterns were generated.

Since the early 1960s, we have studied numerous enzymes of the propionic acid bacteria, and we have found a number of enzymes which are very interesting and unique.[4,5] Transcarboxylase was the first of this series of enzymes which we isolated. This was in 1961, and this enzyme even now continues to intrigue us day by day. I'll confine my comments to transcarboxylase even though some of the other enzymes still occupy our attention.[5] I should note that in spite of our work at the enzymatic level, some aspects of the propionic acid fermentation still remain puzzling.

INTRIGUING ASPECTS OF TRANSCARBOXYLASE

First of all, transcarboxylase is an exception among the biotin enzymes. In general, biotin enzymes catalyze reactions involving carbon dioxide (either fixation of CO_2 or decarboxylation to CO_2), but carbon dioxide has no role in the transcarboxylase reaction. Transcarboxylase catalyzes the following reactions:

$$CH_3\cdot\underset{\displaystyle COO^-}{\underset{|}{CH}}\cdot COSCoA + \text{E-biotin} \rightleftharpoons CH_3\cdot CH_2\cdot COSCoA + \text{E-biotin } COO^- \qquad (1)$$

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$$\text{E-biotin COO}^- + \text{CH}_3\cdot\text{CO}\cdot\text{COO}^- \rightleftharpoons \text{E-biotin} + \text{OOC}\cdot\text{CH}_2\cdot\text{CO}\cdot\text{COO}^- \quad (2)$$

The sum of Reactions 1 and 2 is

$$\text{CH}_3\cdot\underset{\displaystyle\text{COO}^-}{\underset{|}{\text{CH}}}\cdot\text{COSCoA} + \text{CH}_3\cdot\text{CO}\cdot\text{COO}^- \rightleftharpoons \text{CH}_3\cdot\text{CH}_2\cdot\text{COSCoA} + {}^-\text{OOC}\cdot\text{CH}_2\cdot\text{CO}\cdot\text{COO}^- \quad (3)$$

As with all other biotin enzymes, the mechanism consists of two partial reactions. In the first partial reaction, the carboxyl group is transferred from methylmalonyl-CoA to a biotinyl group on the enzyme (E), yielding the carboxylated enzyme and propionyl-CoA. With other biotin enzymes which catalyze fixation of CO_2, the carboxylation of biotin involves ATP and CO_2. In the second partial reaction, the carboxyl is transferred to pyruvate to yield oxalacetate. This second partial reaction is just the same as occurs with the biotin enzyme, pyruvate carboxylase, so these two enzymes have much in common. The overall reaction is methylmalonyl-CoA plus pyruvate yields propionyl-CoA plus oxalacetate.

The second intriguing aspect of transcarboxylase is its roles in the propionic acid fermentation. An abridged outline of the mechanism of the fermentation is presented in Figure 1.* Lactate (or glucose via glycolysis) is converted to pyruvate. Then, the transcarboxylase comes into play. There is a catalytic amount of methylmalonyl-CoA in the cell, and transcarboxylase catalyzes the transfer of the carboxyl group from it to pyruvate, giving rise to oxalacetate and propionyl-CoA. Propionate is formed in the fermentation by this mechanism. The oxalacetate in turn is reduced by a series of reactions to succinate. The CoA is transferred from the propionyl-CoA to succinate by another enzyme (CoA transferase), giving rise to succinyl-CoA and the propionate. Then, another interesting enzyme, methylmalonyl-CoA mutase, which contains vitamin B_{12} comes into play. It catalyzes the converstion of the succinyl-CoA to methylmalonyl-CoA. Thus, the cycle is completed, and the methylmalonyl-CoA is regenerated and is there to react with the next molecule of pyruvate. This whole series of reactions is required to reduce the keto group of pyruvate to the methylene group of propionate. One of the intriguing aspects of biochemistry is to learn the mechanisms nature has devised for reactions. This is a very different process than we would use as chemists. Accompanying this reduction, the pyruvate is oxidized to acetate and CO_2.

The third and probably the most interesting aspect of transcarboxylase is its complex structure. Why this complex structure is required to catalyze a rather simple reaction is still to be answered. A stylized model of the enzyme is shown in Figure 2. It is seen that transcarboxylase is made up of three subunits:

The first type is the central subunit. Each of the cylinders represents a polypeptide, and there are six in the central subunit. It is a hexamer of identical polypeptides. It has a sedimentation coefficient of 12S, and we call it the $12S_H$ or central subunit. The H stands for head. We will see later why we call this subunit the head.

The second type of subunit is called the $5S_E$ subunit or outside subunit. The sedimentation coefficient is 5.9S, and the E stands for ear. There are three of these subunits on each end of the central $12S_H$ subunit. Each of the $5S_E$ subunits consists of two identical polypeptides.

The third type of subunit is shown as the small tubes. It is called the biotinyl or $1.3S_E$ subunit, and the biotins are shown as small hexagons. There are 12 of these biotinyl subunits. Most of them can't be seen in the model.

The entire enzyme is made up of 30 polypeptides, and it has a molecular weight of 1.2 million.[6] It has a sedimentation coefficient of 26S. We, therefore, call this form of the enzyme the 26S enzyme.

* All figures appear following the text.

We shall see that the biotinyl subunits have two functions:

1. They provide the linkage between the central and outer subunits.[7] Part of the polypeptide binds to the central $12S_H$ subunit and a part to the outside subunit. In this respect, transcarboxylase is different from enzymes. Most enzymes have compact and rather rigid structures, but transcarboxylase has a flexible structure. The variable structure occurs because the biotinyl subunit is a flexible polypeptide, and this allows the outside subunits to assume different positions and distances relative to the central subunit.
2. The second function of the biotinyl subunit is to serve as a carboxyl carrier. One of the partial reactions occurs on the central subunit and the other on the outside subunit, and the biotin shuttles back and forth carrying the carboxyl group from the first partial reaction on the central subunit to the second partial reaction on the outside subunit.[8]

One of the most interesting structural features of transcarboxylase is that it dissociates with the loss of three outside subunits exclusively from one face of the central subunit (Figure 3).[9] Why the outside subunits come off of just one side rather than randomly from both sides is intriguing. It may indicate that the two faces of the central or $12S_H$ subunit differ structurally. If so, this has some very important implications concerning the structure of the central subunit. We'll have more to say about this later. The enzyme with three attached subunits is active, and we call it the 18S form, since its sedimentation coefficient is 18S. This dissociation to the 18S form occurs very rapidly at neutral pH. In fact, for about 12 years, we worked with the 18S form and thought it was the native or natural form of the enzyme. Now we know if special precautions are taken and the pH is kept somewhat acid, the intact 26S form of the enzyme can be isolated from the bacteria.[6]

The $5S_E$ subunit dissociates form the 26S enzyme with the biotinyl subunit still attached as shown in Figure 3. This combination is called the $6S_E$ subunit.

Now, I'd like to give some indication of why we think the structure of transcarboxylase is as I have shown it to you and the role each subunit plays in the catalysis.

I'll say a few words about:

1. The use of electron microscopy to study the structure
2. The evidence that each subunit has a separate function
3. The use of trypsin to study how the subunits are linked together
4. The determination of the amino acid sequence involved in linking the subunits together
5. The studies of the structure of the $12S_H$ subunit

ELECTRON MICROGRAPHS OF TRANSCARBOXYLASE

The proposed model of the structure of transcarboxylase is based on a large body of facts, but an important part of the evidence has been obtained by electron microscopy which allows visualization of the structure. Figure 4 is one of our early electron micrographs of the 18S form of the enzyme. At a glance, you can see there are many different profiles. This is because the enzyme is labile and dissociates during preparation of the grids for the electron microscope. Present are outside subunits, central subunits, and central subunits with one, two, or three outside subunits attached.

Profiles of Figure 4 designated by a and b illustrate why the enzyme got the nickname, Mickey Mouse. As you can see, they look like a head with two ears. The ears

or outside subunits are loosely attached, and they are seen at variable distances from the head or central subunit. This profile is a side or edgewise view of the molecule and presents the end-on view of the outside subunits. For some reason, the free enzyme absorbs to the grid in this position. Later, you will see the top view of the molecule and the hole in the central subunit and the lengthwise view of the outside subunits. One of the outside subunits of the 18S enzyme has been lost in the Mickey Mouse form. The full complement of three outside subunits of the 18S form are apparent in the profiles marked a. You see the ears are all located on one side of the head.

An electron micrograph[9] of the 26S form of the enzyme is shown in Figure 5. In this case, the enzyme was treated with a cross-linking reagent (carbodiimide) so it did not dissociate. You can see that the profiles are quite different than those of Figure 4. There are outside subunits on both faces of the central subunit. Usually, three distinct outside subunits are not seen on each opposite face, but if one examines the model (Figure 2), it becomes obvious that some subunits will be hidden by those in front. The main point is that there are subunits on both faces, and from other information we know, there are 6 of them and 12 biotins.[6]

The most informative electron micrographs were obtained through use of avidin (Figure 6). These experiments were done with the 18S form of the enzyme.[10] Avidin combines very strongly with biotin and serves as a marker for the biotin. Two features became evident from the profiles of the avidin complexes. First of all, the avidin complex absorbs to the grid, so a top view of the enzyme is observed. Now, the hole in the central subunit becomes evident. Furthermore, the lengthwise view of the outside $5S_E$ subunit is seen instead of the dense circular end-on view. Between each of the outside subunits are the smaller avidin molecules. At the left, you see a specimen with two outside subunits. In this case, there are three avidin molecules, one between the two outside subunits and one at each end. Avidin is tetravalent, and it is considered that the avidin between the two subunits may have reacted with biotin from each of the two subunits. These results indicate that there are two biotins on each outside subunit, and the biotins are located near the ends.

FUNCTIONS OF THE INDIVIDUAL SUBUNITS

Now, I would like to fill you in on some of the evidence showing how this enzyme works. The beauty of this enzyme is that it can be dissociated to its subunits and the subunits isolated. Thus, the activity of the individual subunits can be studied. Furtheremore, the active enzyme can be reconstituted from the isolated subunits.[11]

The catalytic activity of the individual isolated subunits is illustrated in Reactions 4 and 5.

$$CH_3\cdot\overset{\overset{COO^-}{|}}{C}H\cdot COSCoA + \text{biotin subunit} \underset{}{\overset{12S_H\ \text{subunit}}{\rightleftharpoons}} CH_3\cdot CH_2\cdot COSCoA + {}^-OOC\text{-biotin subunit} \quad (4)$$

$$^-OOC\text{-biotin subunit} + CH_3\cdot CO\cdot COO^- \underset{}{\overset{5S_E\ \text{subunit}}{\rightleftharpoons}} \text{biotin subunit} + {}^-OOC\cdot CH_2\cdot CO\cdot COO^- \quad (5)$$

The sum of Reactions 4 and 5 is

$$CH_3\cdot\overset{\overset{COO^-}{|}}{C}H\cdot COSCoA + CH_3\cdot CO\cdot COO^- \rightleftharpoons CH_3\cdot CH_2 COSCoA + {}^-OOC\cdot CH_2\cdot CO\cdot COO^- \quad (3)$$

The central $12S_H$ subunit in combination with methylmalonyl-CoA catalyzes one partial reaction, i.e., the carboxylation of the biotinyl subunit with formation of pro-

pionyl-CoA. The outside $5S_\varepsilon$ subunit specifically catalyzes the other partial reaction, i.e., transfer of the carboxyl from the biotinyl subunit to pyruvate to form oxalacetate. If the $5S_\varepsilon$ subunit is substituted for the $12S_H$ subunit or the $12S_H$ subunit for the $5S_\varepsilon$ in these partial reactions, no reaction occurs.[8]

The combination of these partial reactions gives the overall reaction. However, the overall reaction is extremely slow with the isolated unassembled subunits. When the subunits are unassembled, the biotinyl subunit must diffuse from one subunit to the other to act as a carboxyl carrier, whereas, when the isolated subunits are combinaed in the form of the intact enzyme, the biotin is so oriented that it is in extremely close proximity to the active sites on the $12S_H$ subunit and the $5S_\varepsilon$ subunit.

Mildvan and co-workers[12-14] by NMR and EPR studies with transcarboxylase have presented evidence that the propionyl-CoA is only about 8 Å from pyruvate (Figure 7). Since, as we have seen, the CoA ester site is on the central subunit and the keto acid site is on the outside subunit, these subunits must be very close together. Since the biotin transfers carboxyls between these sites, it probably is situated between them, perhaps about 4 Å from each. We have thought of the biotin as flip-flopping back and forth between the sites; it may be more like an oscillation or direct transfer. This structural organization is a splendid example of why enzymes are such excellent catalysts. As noted, if the subunits are unassembled, the reaction occurs by diffusion only. In the assembled complex, all the active parts are positioned so that there is almost direct transfer.

Now, I would like to describe how trypsin has been used to prove that one part of the biotinyl subunit is involved in binding together the central and outside subunits and another portion provides for placement of the biotin so that it serves as the carboxyl carrier.

We have found, as illustrated in Figure 8, that when transcarboxylase is treated with trypsin, a portion of the biotinyl subunit containing the biotin (indicated by the hexagon) is cleaved from the enzyme yielding biotinyl peptides, but the main subunit complex remains intact. The complex is inactive, of course, as an enzyme because it does not contain biotin. When this typsinized enzyme is placed on a Bio-Gel® column, these biotinyl peptides can be separated from the residual transcarboxylase complex.[7] There are two major biotinyl peptides, one consisting of 65 amino acid residues and a second consisting of 50 residues. We will see that these two biotinyl peptides will be used in the amino acid sequence studies to be discussed later.

The isolated trypsinized enzyme can now be used to isolate the remaining portion of the biotinyl subunit which holds the outside and central subunits together. The trypsinized transcarboxylase is dissociated at pH 8 to the $12S_H$ subunit and to the outside subunits to which the remaining portion of the biotinyl subunit is still attached, designated as $6S^-_\varepsilon$ in Figure 8. These two subunits are then separated on Bio-Gel®. The isolated $6S^-_\varepsilon$ subunit is then dissociated at pH 9, and the resulting $5S_\varepsilon$ subunit and the portion of the biotinyl subunit which serves in the binding are separated by Bio-Gel® chromatography.[7] We call this portion of the biotinyl subunit, the non-biotinyl peptide.

These separate fractions of the biotinyl subunit have been used to determine the respective functions of the biotinyl subunits. The experiment illustrated in Figure 9 provides the evidence that the nonbiotinyl portion of the biotinyl subunit serves to bind together the central $12S_H$ and outside $5S_\varepsilon$ subunits. Ultracentrifuge patterns are shown which were obtained when isolated subunits were treated in acetate buffer at pH 5.5 to cause their recombination.[7] The first frame shows the results with a mixture of only the $5S_\varepsilon$ and $12S_H$ subunits. Sedimentation is from right to left, and only two peaks were observed, one at ~5S and the other at ~12S. The next frame shows the results when the intact $1.3S_\varepsilon$ biotinyl subunit is added in combination with the $5S_\varepsilon$ and

$12S_H$ subunits; a third peak is observed which is the reconstituted enzyme. Enzymatic analysis showed that active enzyme is formed from the inactive subunits in this preparation. The next frame shows that similar results are obtained when the nonbiotinyl peptide is substituted for the complete $1.3S_E$ subunit. Thus, it is clear that the nonbiotinyl peptide has the information required to cause combination of the $5S_E$ and $12S_H$ subunits and yields the combined form. The resulting protein complex is inactive, of course, because it does not contain the biotin.

When the 65-residue or the 50-residue portions of the biotinyl subunit, which actually contain the biotin, are tested in this system, they do not cause combination of the $12S_H$ and $5S_E$ subunit. Thus, this portion of the biotinyl subunit does not contain the amino acid sequence which serves to tie the subunits together. However, either of these two peptides are as effective as the intact $1.3S_E$ biotinyl subunit in Partial Reactions 4 and 5 which we have discussed previously. However, free biotin, biocytin, or simple derivatives of biotin do not substitute for the biotinyl peptides in these partial reactions.[8] Thus, it is evident that the amino acid sequence surrounding the biotin is important in orienting the biotin in the proper positions on the $12S_H$ and $5S_E$ subunits so that the partial reactions occur.

AMINO ACID SEQUENCE OF THE BIOTINYL SUBUNIT

Clearly, it would be very interesting to know the amino acid sequences which are required for these different functions of the individual portions of the biotinyl subunit, and I'd now like to turn to these studies. The strategy we have used in determining the sequence is shown in Figure 10. The intact biotinyl subunit and three peptides derived from it were used.[15] They were the 65-residue and 50-residue biotinyl peptides which were obtained as explained previously by treatment of transcarboxylase with trypsin, and the third was a peptide obtained by cyanogen bromide cleavage. Using the complete biotinyl subunit, 55 residues of the total of 123 residues were sequenced. With the 65-residue biotinyl peptide, 23 residues were sequenced, and the first 12 residues matched the last 12 of the 55 found using the intact biotinyl subunit, thus proving its location in the intact suunit. With the 50-residue biotinyl peptide, 41 residues were sequenced, and the first 3 residues matched the last 3 of the known 55-residue sequence of the biotinyl subunit, and the first 13 residues matched the last 13 of the 65-residue biotinyl peptide. Thus, its location was established. The peptide obtained by cyanogen bromide cleavage was used to sequence the carboxyl terminal portion.

The complete sequence as known at the present time is shown in Figure 11. As you can see, there are some question marks here and there, and we still need to determine the last ten residues at the carboxyl terminal.*

Let us now look at portions of this sequence in more detail. The sequence of the nonbiotinyl peptide which we have seen binds together the central $12S_H$ and outside $5S_E$ subunits is shown in Figure 12. It consists of the sequence from 1 to 42. Since the intact enzyme dissociates to its subunits at alkaline pH, it seems likely that ionic charged groups are involved in the binding. Viewed in this light, there are two regions which are attractive as possible combining regions. They are the repeating aspartyl and valyl residues between residues 12 and 20, as well as the glutamyl and aspartyl groups at residues 23 and 24. In addition, the alternating hydrophobic and basic residues from one to five also provide possibilities for combining forces. It is likely that one portion combines with the central subunit and another with the outside subunit, and the residues between allow for the flexible space between the subunits. It will be extremely

* The complete sequence has now been determined: See Maloy, W. L., Bowien, B. U., Zwolinski, G. K., Kumar, K. G., Wood, H. G., Ericsson, L. H., and Walsh, K. A., *J. Biol. Chem.*, in press, 1979.

interesting to cleave the 42-residue nonbiotinyl peptide into smaller peptides and to determine which portion is essential for combination with the central subunit and which portion for combination with the outside subunit.

The sequence around the biotinyl portion of the biotin enzymes is of special interest, since this is the portion of the enzyme which is most likely to be conserved in different biotin enzymes during evolution of the species. Keech and collaborators[16] in Australia have determined the sequence surrounding the biotin of pyruvate carboxylase from sheep, chicken, and turkey, and Bradshaw and collaborators[17] of Washington University, the sequence of acetyl-CoA carboxylase of *Escherichia coli*. A comparison of these sequences is shown in Figure 13. It is noted that in the case of pyruvate carboxylase, the sequence is identical in the chicken and turkey enzymes, and these differ from the sheep enzyme only in three positions: where a Glu is substituted for Ala at position 2 and a Ser for an Ala at position 17 and a Val for an Arg at position 19. Thus, there is almost complete conservation of the sequence between the avian and animal enzymes. You will note, too, that the sequence for transcarboxylase is quite similar to that of pyruvate carboxylase, the identical residues are blocked off. It might be expected that pyruvate carboxylase and transcarboxylase would have similarity since the second partial reaction (Reaction 2), the carboxylation of pyruvate, is identical for the two enzymes. The sequence of acetyl-CoA carboxylase is similar, too, but it differs much more than does transcarboxylase from pyruvate carboxylase, but near the biotin, it is identical.

It is believed that bacteria diverged from the eukaryotes almost 3000 million years ago. Yet, this sequence appears to have been conserved and, therefore, must be very essential in the reaction by biotin enzymes.

STRUCTURE OF THE CENTRAL SUBUNIT

Now, we shall turn to a consideration of the structure of the central $12S_{\prime\prime}$ subunit. It has very interesting properties. You recall that it is made up of 6 polypeptides, but there are 12 biotinyl subunits attached to it, thus, 2 per polypeptide of the central subunit. To provide for these two sites, we have proposed[10,18] that there may have been gene duplication and fusion which resulted in a duplicating amino acid sequence in the polypeptides. The resulting homologous sequence as represented by the dashed and solid lines is shown in Figure 14. Thus, two binding sites could be provided per polypeptide. The two portions of the sequence need not be identical, so the binding sites could differ, one represented by the solid circles (B), the other by the hatched circles (B′). Not only would the homologous sequence provide for dual binding sites for the biotinyl subunit, but it also could provide for dual sites for the CoA ester substrates, i.e., 12 CoA ester sites, 1 for each biotin. All 12 biotins are carboxylated.[6]

We have attempted to determine if there is a duplication within the sequence by tryptic peptide mapping.[19] However, by this technique, no evidence of homology was found. Within experimental error, the number of peptides was equal to the sum of the arginines plus lysines plus one. However, this finding by no means eliminates the possibility that there is homology. Following gene duplication and fusion, mutations may have occurred which destroyed the exact identity of the two portions of the homologous sequence. In fact, it has been possible in only a few enzymes, for example, in RNA synthetases,[20] to demonstrate homology by peptide mapping.

We, therefore, have turned to another procedure. If there is homology, there probably would be 2 CoA ester sites per polypeptide or 12 per $12S_{\prime\prime}$ subunit. For these studies, a photoaffinity label, para-azidobenzoyl-CoA (PABCoA), was used as an analog of the CoA ester as outlined in Figure 15. ^{14}C-PABCoA was mixed with transcarboxylase at 0°C for a few minutes and then exposed to light which causes the nitrene

to form which reacts covalently at the CoA ester site and inactivates the enzyme.[21] A portion of the mixture was then assayed for enzymatic activity, and the remainder was passed through an ultra filter. The radioactivity bound to the enzyme, which was retained by the filter, was then determined. Knowing the total radioactivity and the specific radioactivity of the PABCoA, the nanomoles of PABCoA bound by the enzyme could be calculated.

The loss in enzymatic activity plotted against the nanomoles of PABCoA bound per nanomole of enzyme is plotted in Figure 16. It is seen that extrapolation to zero enzymatic activity gives a value of 12.8 nmol of PABCoA bound per nanomole of enzyme. Extrapolation is required, since at high concentrations of PABCoA, there is nonspecific binding to the enzyme, and there no longer is a linear relationship of the amount of bound PABCoA and the activity of the enzyme. When CoA or methylmalonyl-CoA is included along with the PABCoA, they act as competitive inhibitors of the photoaffinity label, thus indicating the binding of PABCoA is specific for the CoA ester sites.[21] These results indicate there are two binding sites per polypeptide of the $12S_H$ subunit as expected if there is homology. It is planned in future experiments to treat the PABCoA-labeled polypeptide with trypsin, isolate the resulting labeled peptides (hopefully two), and sequence them. In this way, we hope to demonstrate homology in the amino acid sequence at the CoA ester sites. This result would provide direct support for the proposal of gene duplication and fusion.

Finally, there is a question of the arrangement of the six polypeptides in the central subunit. You recall the outside subunits of the 26S enzyme dissociate exclusively from one face of the $12S_H$ subunit when it is converted to the 18S form. In the electron microscope, one seldom, if ever, sees an 18S enzyme with two outside subunits on one face and one on the other face as would occur if the dissociation was random from the two faces.

Two explanations come to mind.

First, the structures of the two faces differ. This could occur as illustrated by the model on the left of Figure 17. In this case, the six polypeptides are arranged in a parallel manner so that one type of binding site is exclusively on one face (solid circles) and the other type (hatched circles) is on the other face. The binding properties of the two faces would thus differ, and preferential dissociation could be expected from the two faces.

The second possibility is that the faces are identical as illustrated by the model on the right of Figure 17 in which there is an antiparallel arrangement of the polypeptides. In this case, the binding sites are the same on both faces, which is indicated by the three solid and three hatched circles on both faces. In this situation, it is necessary to invoke an explanation such as cooperativity to account for the results. For example, the loss of an outside subunit from one face (no matter which face) might cause a conformation change and facilitate the loss of the other outside subunits from that same face, but strengthen the binding of the outside subunits on the other face.

We have attempted to differentiate between these two possibilities by exchanging ^{60}Co-labeled outside subunits with 26S transcarboxylase and then dissociating the resulting labeled 26S transcarboxylase to 18S transcarboxylase.[19] I will not attempt to present the results in detail. I will only say that the exchange was not random on the two faces, which supports the proposition that the central subunit has different structures on the two faces and a parallel arrangement of the polypeptides. We hope to crystallize the $12S_H$ subunit and verify this conclusion by X-ray crystallography.

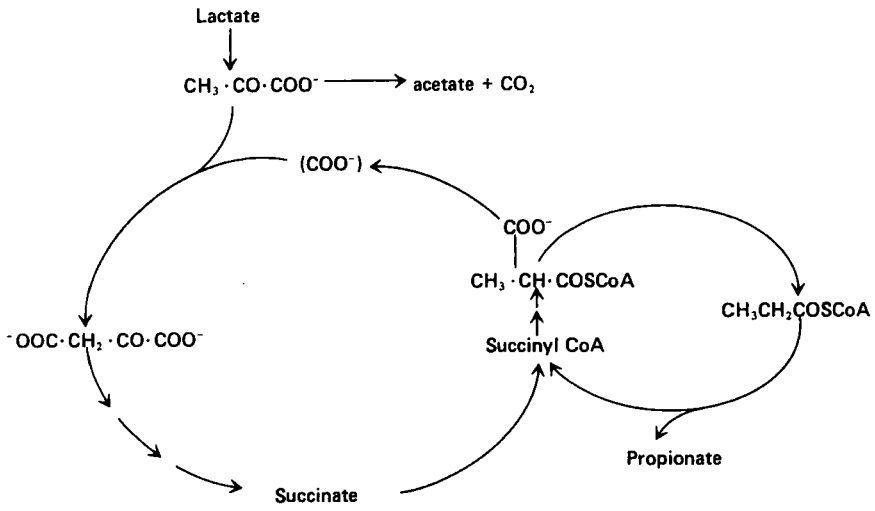

FIGURE 1. An abridged scheme showing the role of transcarboxylase in the mechanism of the propionic acid fermentation.

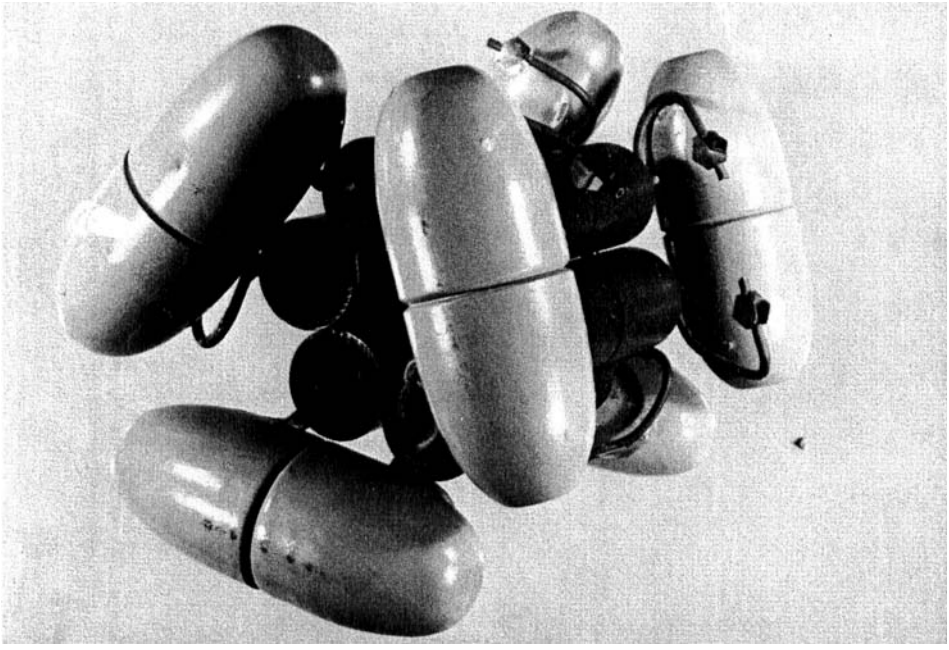

FIGURE 2. Model of the 26S form of transcarboxylase. The $1.3S_\epsilon$ biotinyl subunits are represented by the tube-like structures which bind the light colored dimeric outside $5S_\epsilon$ subunits to the darker colored central hexameric subunit. The hexagons represent the biotins of the biotinyl subunit.

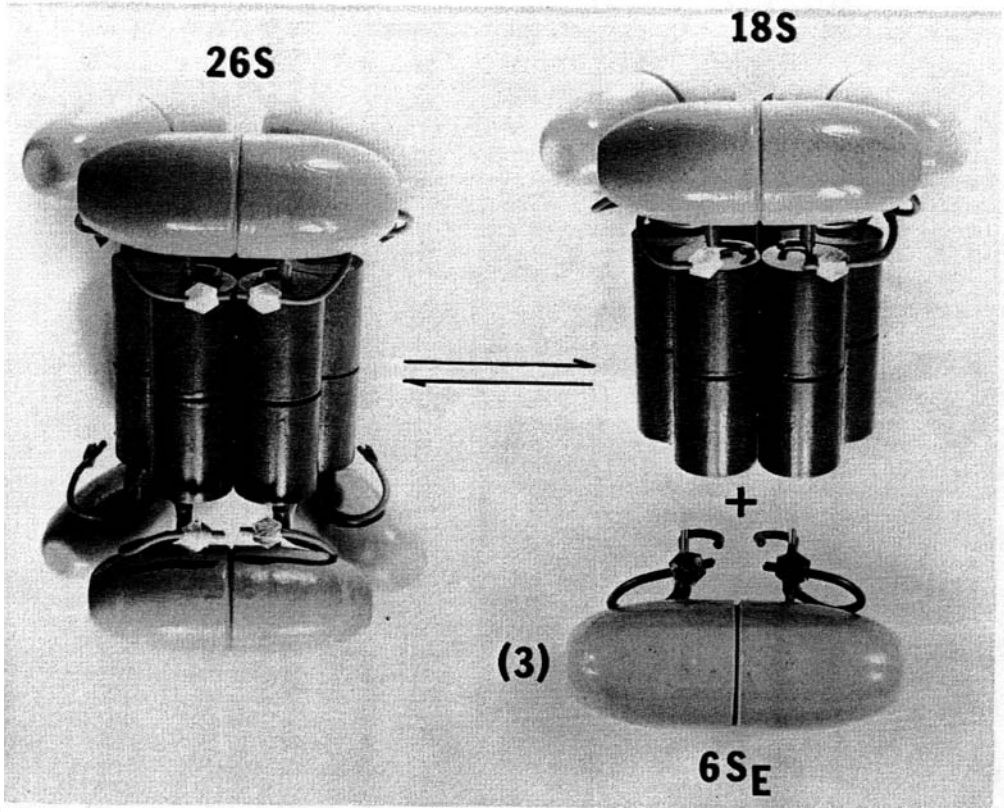

FIGURE 3. Representation by models of the dissociation of 26S transcarboxylase to 18S transcarboxylase and three 6S$_\varepsilon$ subunits. The 6S$_\varepsilon$ subunit consists of the 5S$_\varepsilon$ subunit to which the two 1.3S$_\varepsilon$ biotinyl subunits remain attached.

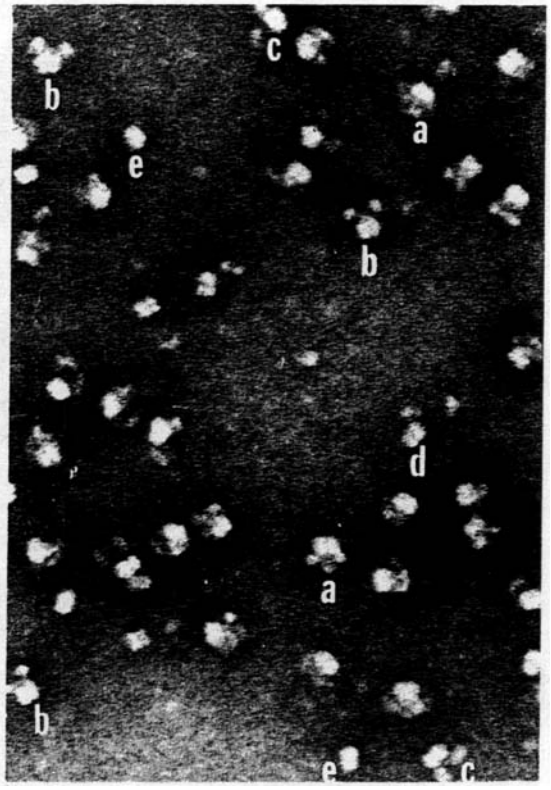

FIGURE 4. Electron micrograph of transcarboxylase (a) 18S form with three outside 5S$_\varepsilon$ sununits, (b) 16S form with two outside 5S$_\varepsilon$ subunits, (c) 16S form with the 5S$_\varepsilon$ subunits partly detached, (d) central subunit with only one 5S$_\varepsilon$ subunit attached, (e) central subunit only.

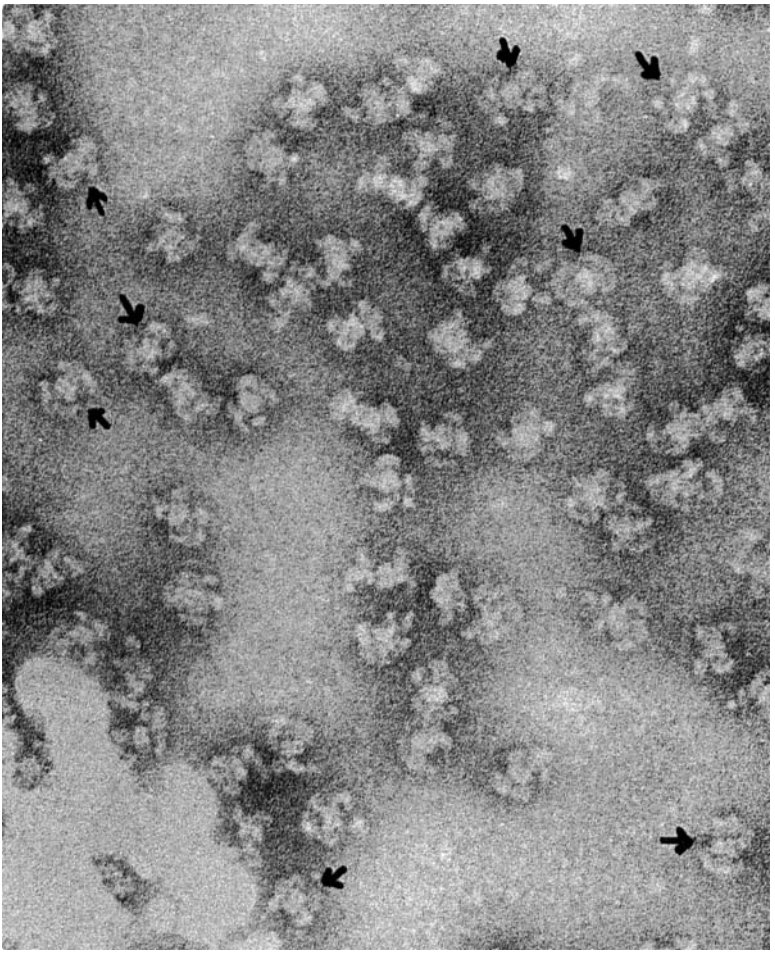

FIGURE 5. Electron micrograph of the 26S form of transcarboxylase which has been cross linked with carbodiimide to prevent dissociation of the outside subunits. Note that the outside subunits are on both faces in contrast to profiles of the 18S form of transcarboxylase (Figure 4). Some of the clearer specimens of 26S transcarboxylase are arrowed.

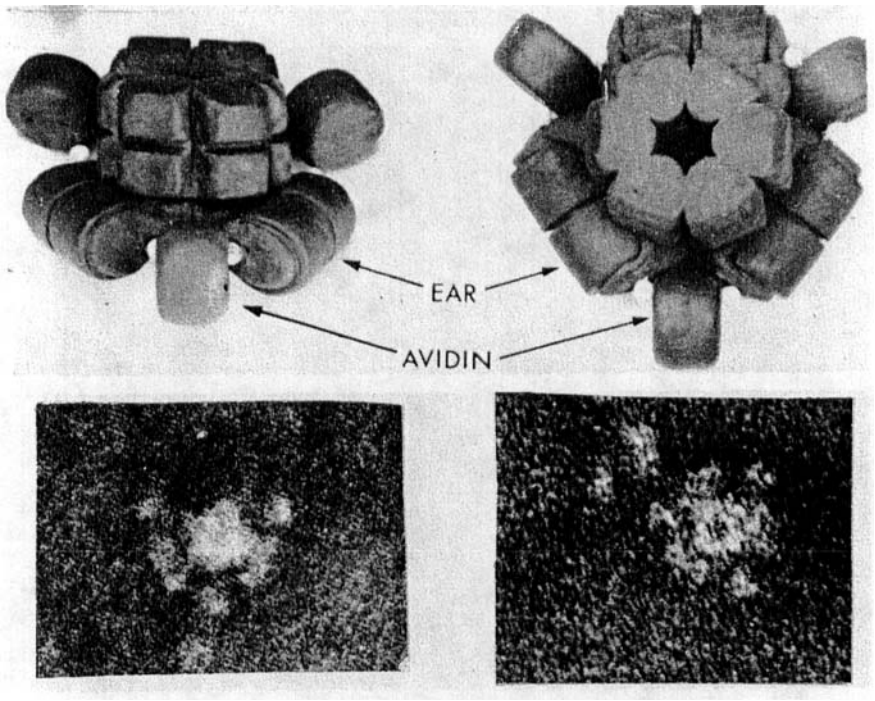

FIGURE 6. Electron micrographs of complexes of avidin with the 16S and 18S forms of transcarboxylase and models proposed to represent the structures.

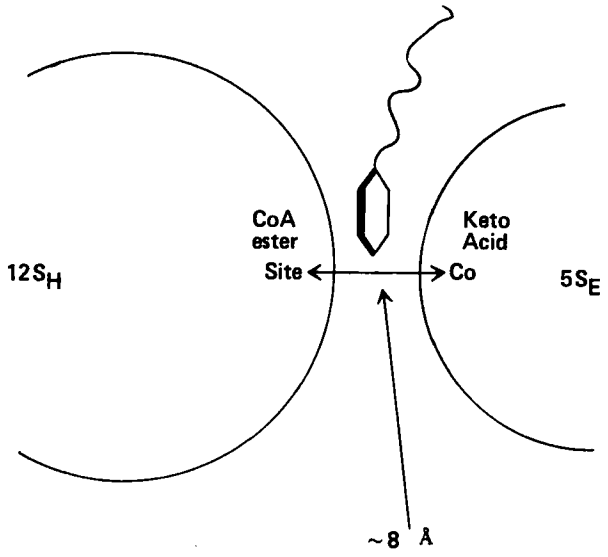

FIGURE 7. Illustration of the distances between a CoA ester site on the 12S$_H$ subunit and a keto acid site on the 5S$_E$ subunit based on measurements by Mildvan and co-workers.[12,13,14] The biotin is indicated by the hexagon.

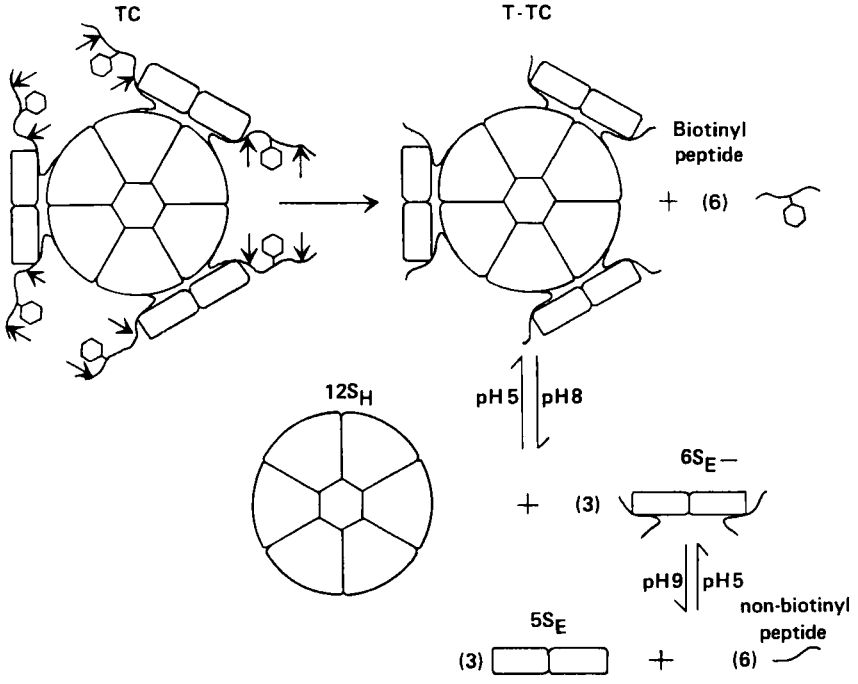

FIGURE 8. Illustration of the action of trypsin on the 18S form of transcarboxylase and the procedure of isolating biotinyl peptides and nonbiotinyl peptides.

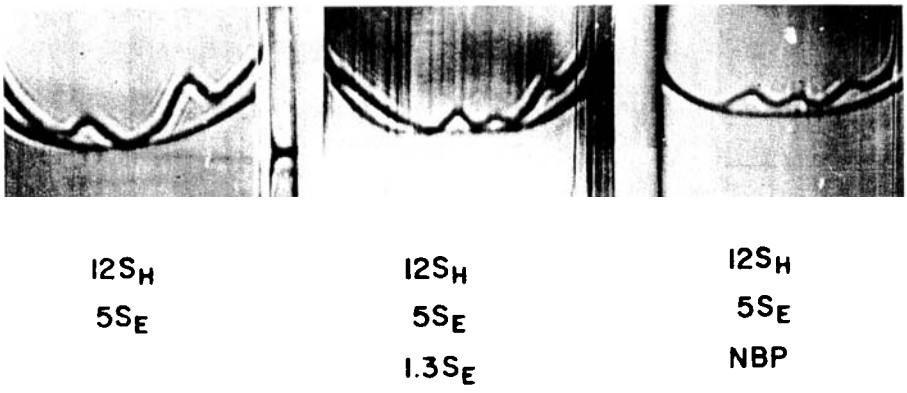

FIGURE 9. Evidence that the 42-residue nonbiotinyl peptide (NBP) provides linkages for combination of the 5S$_\varepsilon$ and 12S$_H$ subunits. Sedimentation is from right to left. Note in the presence of the 1.3S$_\varepsilon$ subunit or nonbiotinyl peptide, a third peak consisting of the complexed 12S$_H$ and 5S$_\varepsilon$ subunitsis present.

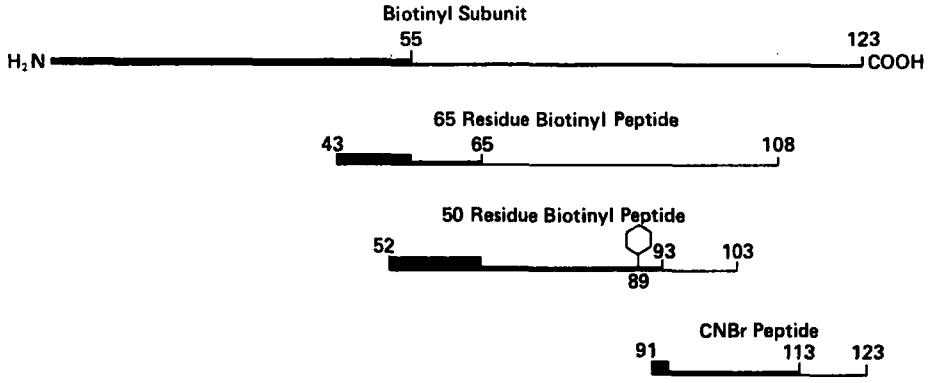

FIGURE 10. Procedure used in determining the amino acid sequence of the $1.3S_\varepsilon$ biotinyl subunit. The heaviest portion of the line indicates the overlap of the sequence determined with a polypeptide compared to the polypeptide shown above it. The next heaviest portion of the line indicates the extent of the sequence determined on the polypeptide, and the overall length of the line indicates the total length of the polypeptide. The 65- and 50-residue biotinyl peptides were obtained as illustrated in Figure 8.

```
1                                   10                                  20
MET-LYS-LEU-LYS-VAL-THR-VAL-ASN-GLY-THR-ALA-TYR-ASP-VAL-ASP-VAL-ASP-VAL-ASP-LYS

21                                  30                                  40
SER-HIS-GLU-ASP-PRO-MET-GLY-THR-ILE-LEU-PHE-GLY-GLY-GLY-?  -GLY-GLY-ALA-PRO-ALA

41                                  50                                  60
PRO-ARG|ALA-ALA-GLY-GLY-ALA-GLY-ALA-GLY-LYS|ALA-GLY-GLU-GLY-GLU-ILE-PRO-ALA-PRO
       |65BP ------>               |50BP ------>
61                                  70                                  80
LEU-ALA-GLY-THR-VAL-SER-LYS-ILE-LEU-VAL-LYS-GLU-GLY-ASP-THR-VAL-LYS-ALA-GLY-GLN

81                                  90                                  100
?  -VAL-LEU-VAL-LEU-GLU-ALA-MET-BCT-MET-GLU-THR-GLU-ILE-ASN-ALA-PRO-THR-SER-GLY
                                                                  <------50BP
101                                 110
LYS|VAL-GLU-LYS-VAL-GLU-LYS|VAL-LEU-GLN-LYS-  ? -ALA
<------|          <------65BP|
```

FIGURE 11. The amino acid sequence of the $1.3S_\varepsilon$ biotinyl subunit as known at present.

```
1                   5                   10
MET-LYS-LEU-LYS-VAL-THR-VAL-ASN-GLY-THR-

11                  15                  20
ALA-TYR-ASP-VAL-ASP-VAL-ASP-VAL-ASP-LYS-

21                  25                  30
SER-HIS-GLU-ASP-PRO-MET-GLY-THR-ILE-LEU-

31                  35                  40
PHE-GLY-GLY-GLY-  ?  -GLY-GLY-ALA-PRO-ALA-

41
PRO-ARG
```

FIGURE 12. The amino acid sequence of the nonbiotinyl peptide which serves to link together the $5S_\varepsilon$ and $12S_w$ subunits.

Enzyme	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Pyruvate carboxylase: sheep	Gly	Glu	Pro	Leu	Val	Leu	Ser	Ala	Met	Bct	Met	Glu	Thr	Val	Val	Thr	Ser	Pro	Val
Pyruvate carboxylase: chicken	Gly	Ala	Pro	Leu	Val	Leu	Ser	Ala	Met	Bct	Met	Glu	Thr	Val	Val	Thr	Ala	Pro	Arg
Pyruvate carboxylase: turkey	Gly	Ala	Pro	Leu	Val	Leu	Ser	Ala	Met	Bct	Met	Glu	Thr	Val	Val	Thr	Ala	Pro	Arg
Transcarboxylase: *P. shermanii*	Gly	?	Val	Leu	Val	Leu	Glu	Ala	Met	Bct	Met	Glu	Thr	Glu	Ile	Asn	Ala	Pro	Thr
Acetyl-CoA carboxylase: *E. coli*	Asn	Thr	Leu	Cys	Ile	Val	Glu	Ala	Met	Bct	Met	Met	Asn	Gln	Ile	Glu	Ala	Asn	Lys

FIGURE 13. Comparison of the amino acid sequence surrounding the biotin in pyruvate carboxylases, transcarboxylase, and acetyl-CoA carboxylase. BCT is biocytin, i.e., a biotin in amide linkage with the ε amino group of a lysine residue.

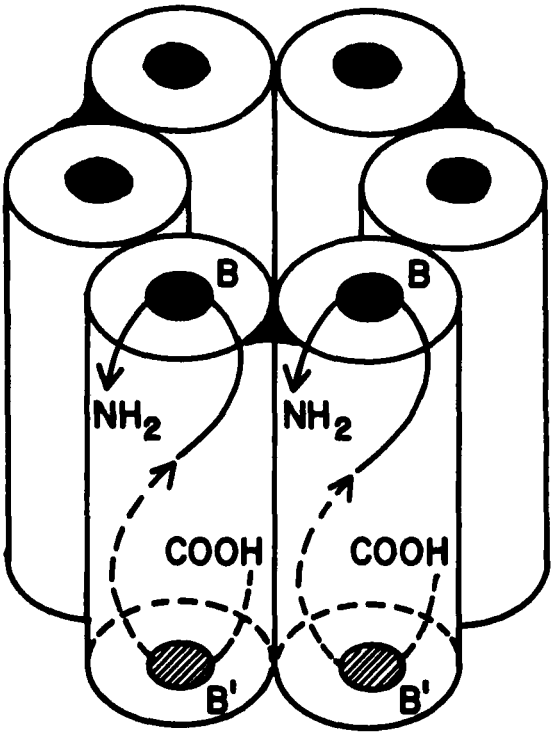

FIGURE 14. Illustration of the $12S_H$ subunit. The possible homology of the sequence is indicated by the solid and dashed lines. B indicates one type of binding site and B′, a similar, but perhaps slightly different binding site for the biotinyl subunits which link the $5S_E$ subunits to both faces of the hexameric $12S_H$ subunit.

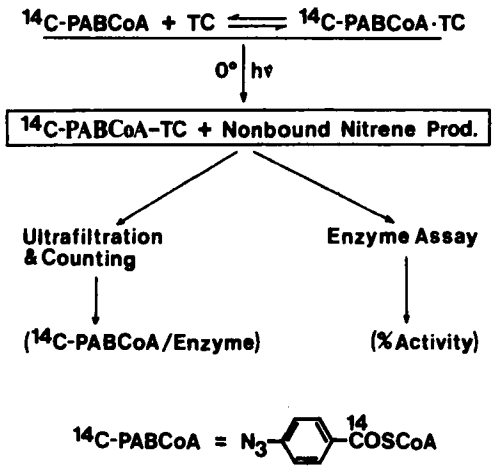

FIGURE 15. Illustration of the procedure used to determine the number of CoA ester sites of transcarboxylase by use of the photoaffinity label [^{14}C]para-azidobenzoyl-CoA (^{14}C - PAB CoA).

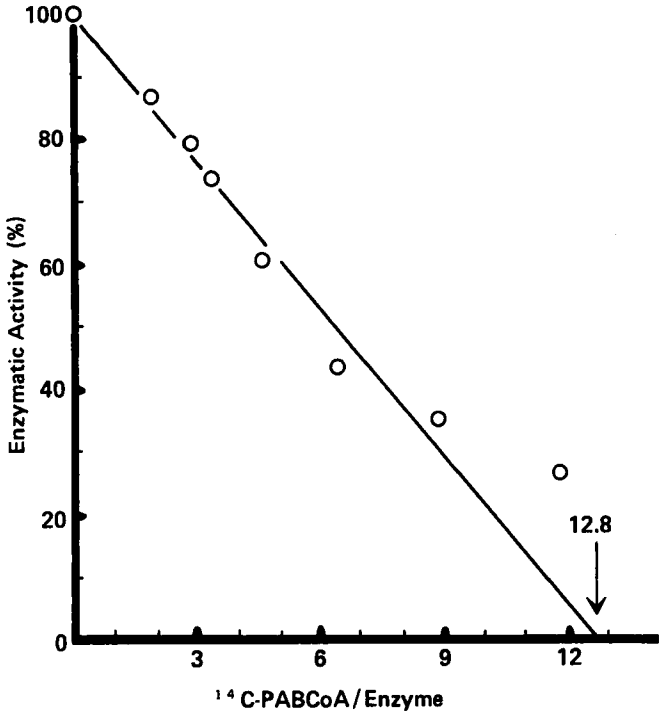

FIGURE 16. Inhibition of the enzymatic activity of transcarboxylase as a function of the number of CoA ester sites blocked by the photoaffinity label, para-azidobenzoyl-CoA (PABCoA).

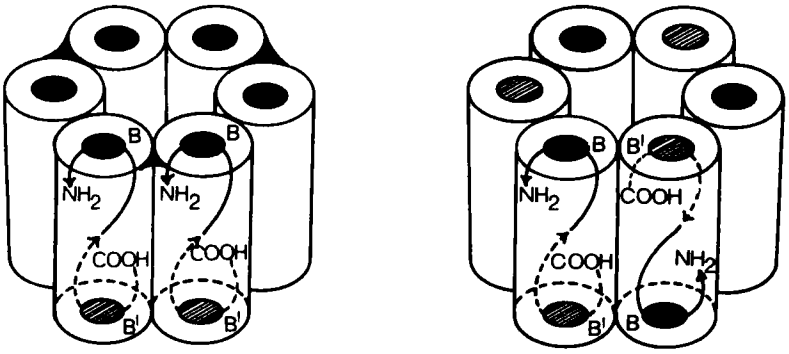

FIGURE 17. Illustration of a parallel arrangement (left) and an antiparallel arrangement (right) of the hexameric central $12S_{\alpha}$ subunit The solid and dashed lines indicate homologous portions of the amino acid sequence. The solid circles indicate one type of binding site for the $1.3S_{\epsilon}$ biotinyl subunit and the hatched circles, a second similar type of binding site.

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