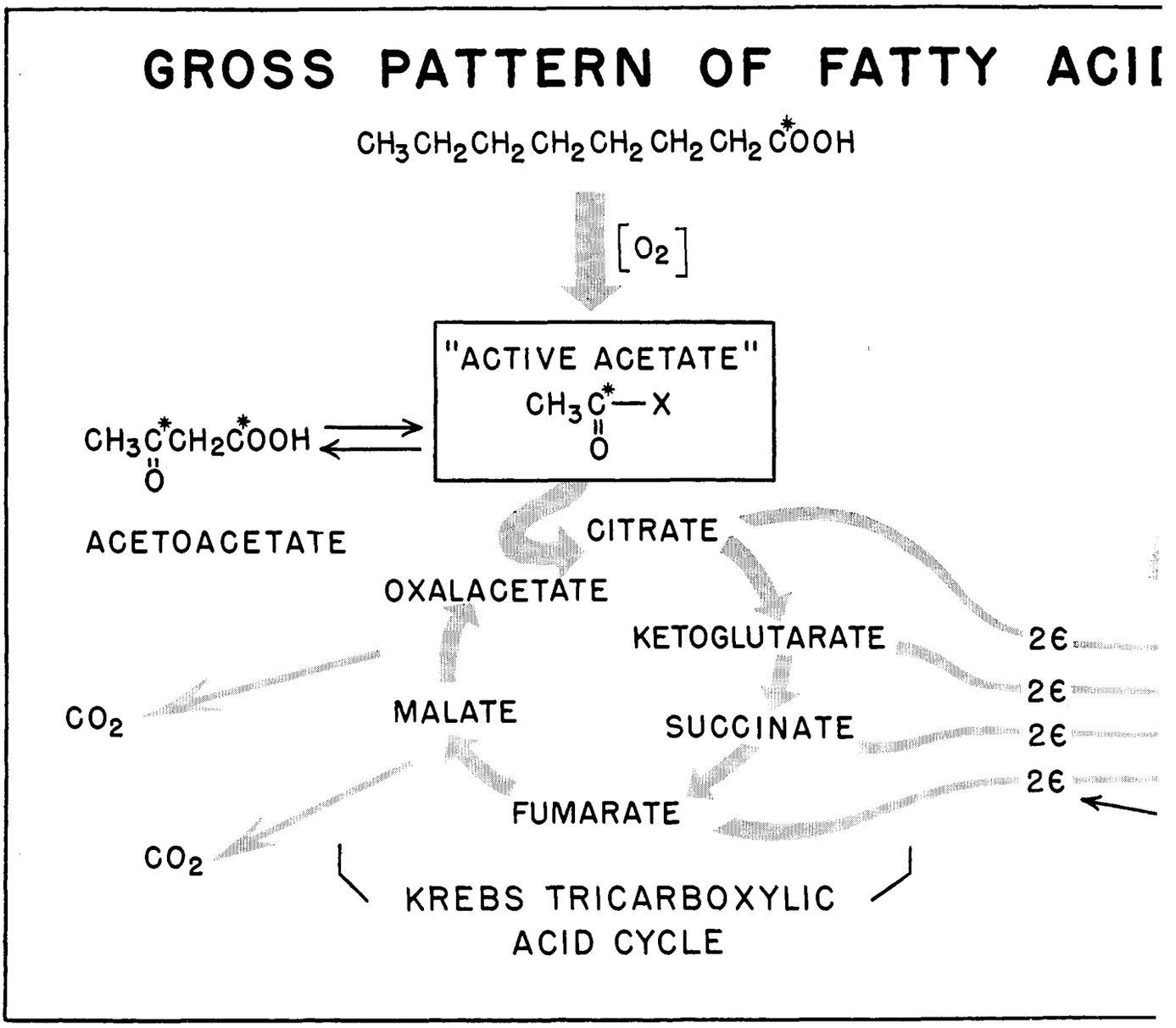

Metabolism Research Findings Enlighten the Path

From Fats to Energy

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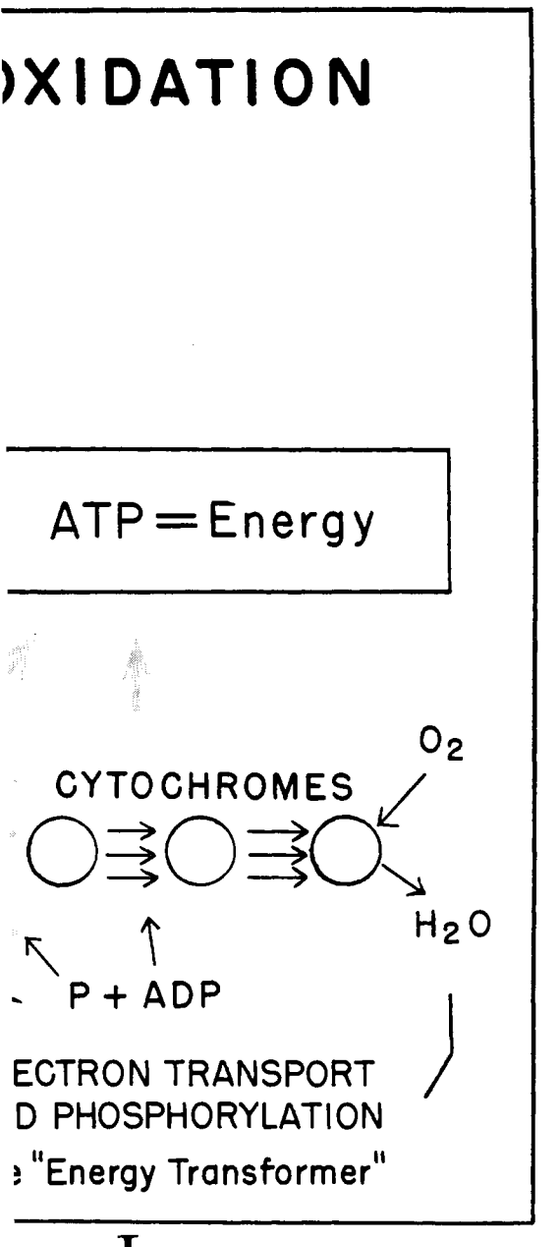
In developing a rational basis for establishing the nature of an "adequate diet," nutritional science must depend heavily on research findings in areas of intermediary metabolism and enzyme chemistry. Recent research has given biochemists and nutritionists a reasonably complete flow-sheet of the mechanism of metabolism of fats. It is expected to be of great importance in understanding complex interrelationships among fats, carbohydrates, proteins, and the various vitamins. Its significance is attested by the 1953 Nobel Award in medicine and physiology to Fritz Lipmann and Hans Krebs, two of the major contributors.



IT IS WELL KNOWN, of course, that fat is an important source of energy in the body, providing about 30 to 40% of the caloric requirement on a "normal" diet. In some conditions, such as fasting or in diabetes, fat may furnish a much larger fraction of the fuel mixture. This is because fat occupies a rather unique role in the organism; it is the only foodstuff which can be stored in large amounts in the animal body and used as an energy source in times of need. The interrelationship between fat and carbohydrate metabolism is therefore a very flexible one which is beautifully adapted to providing a smooth flow of energy to the organism. This complex relationship has been studied for many years but it has been only recently that it could be described on a molecular level; that is to say, in terms of the chemical structure of the intermediate substances involved and the specific enzyme proteins which catalyze the individual steps.

Up to less than 10 years ago, virtually nothing was known about the individual steps involved in the biological oxidation and synthesis of the lipids, whereas a large amount of detailed knowledge concerning carbohydrate metabolism was already available and the intermediary metabolism of amino acids, while not fully understood, was being explored with considerable success. This relative lack of knowledge concerning the intermediary metabolism of fatty acids was certainly not due to lack of effort or interest. Actually, this subject had attracted many outstanding biochemists over the last 50 years because of the importance of fat metabolism in the disease *diabetes mellitus*.

Perhaps the greatest single barrier to identification of the intermediate steps was the fact, first observed many years ago, that an organ such as the liver, which is known to oxidize fatty acids in the intact animal, no longer possessed this ability when the cell structure was destroyed by grinding or extraction. This finding appeared to indicate that the enzymes catalyzing this process could not function in the absence of normal cell structure. This situation obviously made investigation of intermediate steps in fatty acid metabolism very difficult, because in the intact cell the oxidation of fatty acids proceeds smoothly to its end products, carbon dioxide and water, without the accumulation of any significant amount of any of the intermediates in this complex process.

This barrier to research on the intermediary steps in fatty acid oxidation was finally broken in 1944, when it was found by Munoz and by Lehninger that the addition of three substances—namely, Mg^{++}, inorganic phosphate, and adenosine triphosphate (ATP)—would restore the ability of a liver homogenate to oxidize fatty acids. These substances, which are normally present in the tissues but which are diluted when a homogenate of the tissue is prepared, therefore, appear to function as coenzymes in the process of fatty acid oxidation. Experiments soon revealed that the oxidation of fatty acids is catalyzed by particulate elements present in liver cells. When supplemented with the above factors, these particles were found to catalyze the oxidation of octanoate (a short-chain "model" substrate) to two molecules of acetoacetate at the expense of molecular oxygen.

The isotope tracer technique represented another means of deducing the pattern of oxidation of fatty acids. In an important and ingenious experiment, Weinhouse and his colleagues allowed carboxyl-labeled octanoate to be oxidized by liver. The acetoacetate formed was isolated and was found to contain labeled carbon almost equally distributed between the carbonyl- and carboxyl-carbon atoms (Figure 1). This finding demonstrated conclusively that the fatty acid is oxidized by fragmentation into two-carbon units which then recombine at random to form acetoacetate. It ruled out the possibility that the acetoacetate arose by cleavage of the fatty acid directly into four carbon fragments.

Krebs Cycle

Another important advance was made when it was demonstrated that the two-carbon fragments arising from fatty acids have another and more important pathway of metabolism than formation of acetoacetate. It was discovered that these fragments could react with oxalacetate to form citrate and thus enter the Krebs tricarboxylic acid cycle, which is a cyclic enzymatic "mill" already known to be the mechanism by which carbohydrate is oxidized to carbon dioxide and water (via pyruvic acid). The diagram shown in Figure 1 illustrates the over-all pathway of fatty acid oxidation and an outline of the Krebs cycle. The Krebs tricarboxylic acid cycle is thus the final oxidative pathway for all two-carbon fragments derived from fatty acids as well as the final oxidative pathway of carbohydrate. It should be pointed out that in the operation of this cycle, and in the transport of electrons to their ultimate reaction with oxygen, large amounts of energy are liberated and recovered as adenosine triphosphate (ATP), a substance which serves as the cellular energy carrier.

The chemical nature of the active two-carbon fragment then became of great interest. Experiments soon revealed that this was not acetic acid as such, or any other of a variety of two-carbon compounds tested. However, evidence accumulated that acetate could be converted to the active intermediate in the body and the phrase "active acetate" was coined to signify the active or nascent nature of the acetate involved.

Further attempts to learn the chemical

Albert L. Lehninger capped the first 10 years of his scientific career by receiving the Paul Lewis Laboratories award in 1948. After completing a bachelor's degree in chemistry at Wesleyan University, he did advanced work at the University of Wisconsin and received his Ph.D. at Madison in 1942. He continued at Wisconsin until 1945, attaining the rank of assistant professor of biochemistry. He became assistant professor at the University of Chicago, then associate professor in 1949.

In the spring of 1951, Dr. Lehninger went to the University of Frankfurt, Germany, as an exchange professor, thence on Guggenheim and Fulbright fellowships to the University of Cambridge, England, in the fall. Since July 1952, he has been DeLamar professor of physiological chemistry and director of the department at Johns Hopkins school of medicine.

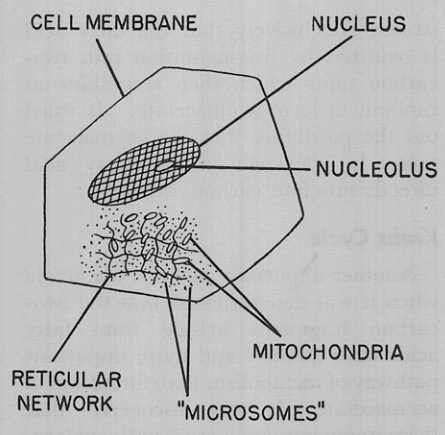


Figure 2. Schematic diagram of a liver cell illustrating some of the intracellular structures

nature of "active acetate" and indeed of other intermediates in the oxidation of fatty acids by study of the cell particles, which catalyzed the over-all process of oxidation, met with little success at first. However, it was recognized that the fatty acid was not oxidized as such but rather underwent some sort of "activation." This activation phenomenon was termed priming or sparking, but its actual nature was quite mysterious. Significantly, there appeared to be a similarity between "active acetate" and the "activated" fatty acid.

Attempts to break up the cell particles catalyzing fatty acid oxidation into separate enzyme fractions, in order to study individual reaction steps, resulted in complete loss of enzyme activity. In addition, these particles showed the striking property of oxidizing fatty acids only when they were freshly prepared and kept in media that were isotonic with body fluids. This finding suggested that the particles were, in fact, subcellular structures with a very definite degree of morphological organization and surrounded by a semipermeable membrane. By means of differential centrifugation, it is possible to isolate from tissue extracts the cell nuclei, smaller cytoplasmic granules called mitochondria, and still smaller elements called microsomes (Figure 2). Since these granular elements are known to be sensitive to osmotic pressure changes, it appeared possible that the fatty acid oxidizing enzymes were localized in one of these structures of the cell. These fractions were isolated and tested for activity in oxidizing fatty acids and it was discovered by Kennedy and Lehninger and by Schneider that the mitochondria (Figure 3) alone possess the ability to oxidize fatty acids. Furthermore, the isolated mitochondria were found to be the intracellular site, not only of fatty acid oxidation, but also of the entire scheme of reactions making up the Krebs tricarboxylic acid cycle. Since these oxidations of fat and carbohydrate represent the main source of energy to the aerobic cell, the mitochondria may be looked upon as the power plants of the cell.

The mitochondria are rod-shaped elements about one to three microns long and contain protein, lipids, and ribonucleic acid. They are not merely tiny sacs containing enzymes in solution, but are highly organized bodies with a very definite fine structure which can be visualized in the electron microscope (Figure 3). If the structure of these bodies is damaged by grinding or by lysis in a hypotonic medium, the complex enzymatic reactions they catalyze lose their organization also. The discovery that fatty acid oxidation occurred in the mitochondria, while of great importance to cytologists, presented the difficult problem of extracting from their structure the individual enzymes concerned in fatty acid oxidation. This was also finally solved but not until the nature of "active acetate" became known.

Chemical Nature of "Active Acetate"

The chemical nature of "active acetate" became clear from the study of certain other enzymatic reactions involving acetate. Lipmann and Nachmansohn and their colleagues had found in 1946 that the enzymatic acetylation of substances such as sulfanilamide and choline required the presence of ATP and a dialyzable, heat-stable substance of coenzyme nature which was called coenzyme A (for acetylation). ATP and CoA were found to be necessary to convert ordinary acetate into "active acetate," which then could combine with an acetate acceptor, such as choline, to form acetylcholine. Coenzyme A was found in all cell types examined. Ultimately, it was learned that it contained, as part of its molecule, the vitamin pantothenic acid. The structure of coenzyme A has been worked out in detail quite recently and is given in Figure 4.

The mechanism by which CoA and ATP convert ordinary acetate into "active acetate" was still obscure even after the structure of the CoA molecule was fairly well known. However, in 1951, Lynen and his colleagues in Munich were able to isolate a small amount of "active acetate" in impure form. The important finding was made that "active acetate" was actually a complex of acetate and coenzyme A. Lynen soon was able to demonstrate that "active acetate" is a thiol ester, in which acetate is esterified to the sulfhydryl group of CoA (Figure 4). CoA may thus be regarded as an acetyl group carrier and the active spot in the CoA molecule is the SH group. In a short time, Lipmann was able to demonstrate that acetyl-CoA is formed from acetate in an enzyme-catalyzed reaction as follows:

(1) adenosine triphosphate + acetate + CoA-SH $\rightleftarrows$ $CH_3C(=O)$-S-CoA (acetyl-CoA) + adenosine monophosphate + inorganic pyrophosphate

This reaction, then, represents the mechanism of formation of "active acetate" from ordinary acetate.

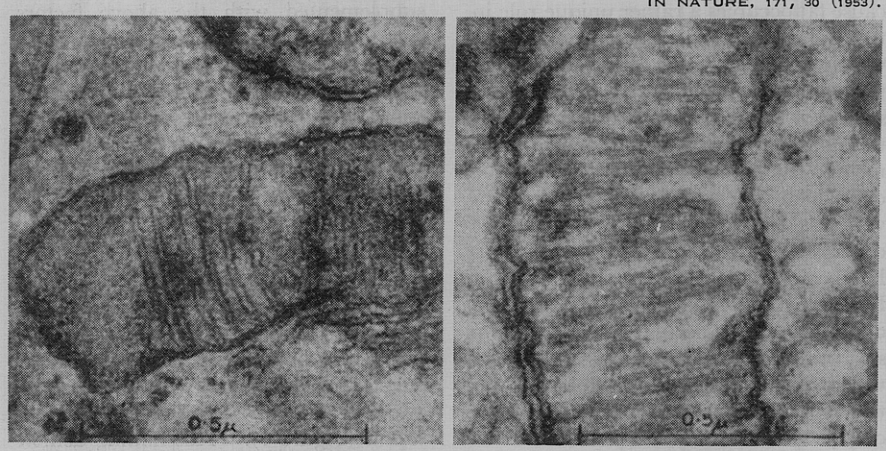
These findings suggested that acetyl-CoA is the active two-carbon intermediate in fatty acid oxidation and it was soon demonstrated in the laboratories of Lipmann and Ochoa that acetyl-CoA took part in the following two important reactions of fatty acid metabolism, each being catalyzed by a specific, highly purified enzyme:

(2) 2 acetyl-CoA $\rightarrow$ acetoacetate + 2 CoA-SH

(3) acetyl-CoA + oxalacetate $\rightarrow$ citrate + CoA-SH

Figure 3. Electron micrographs of ultrathin slices of intact cells, including longitudinal sections through single mitochondria. Note the double limiting membrane, the lamellae or septa running across the body, and the granular fine structure. The many oxidizing and phosphorylating enzyme molecules catalyzing fatty acid oxidation are probably embedded in these structures in a regular and organized spatial arrangement

THESE ELECTRON MICROGRAPHS REPRODUCED FROM THOSE PUBLISHED BY F. S. SJOSTRAND IN NATURE, 171, 30 (1953).



Reaction (2) represents the mechanism of formation of acetoacetate and reaction (3) the mechanism by which acetyl-CoA forms citrate and thus enters the tricarboxylic acid cycle. Acetyl-CoA may therefore be termed an acetyl donor.

This research not only clarified the nature of "active acetate" but also led to the suggestion by Lynen that perhaps long chain fatty acids are enzymatically oxidized, not as the simple carboxylate anions but as the CoA esters (that is, palmityl-CoA, stearyl-CoA). In short, such a mechanism would account for the activation or sparking phenomenon mentioned earlier. Very recently, enzymes have been isolated from mitochondria which do, in fact, catalyze the formation of long chain fatty acid esters of CoA.

(4) RCOOH + ATP + CoA-SH ⇄
RC-S-CoA + adenosine monophosphate
‖
O
+ inorganic pyrophosphate

The enzymatic activation of fatty acids and acetate by formation of CoA esters has been the basic motif of the most recent work on the mechanism of fatty acid oxidation. Within the last year, work in the laboratories of Lynen, Ochoa, Green, Lipmann, Lehninger, and others, has rather definitely established the reaction sequence shown in Figure 5. It can be seen that the fatty acid is first activated to form the CoA ester in which form it is oxidized to the α-β-unsaturated fatty acid - CoA ester. It is then hydrated, with the formation of the β-hydroxy acid - CoA ester. Curiously, the hydroxy acid has the D(−) stereochemical configuration, the opposite of that of the β-hydroxybutyric acid found in the blood and urine in fasting or diabetes. The β-hydroxy acid–CoA ester is then oxidized to the β-keto acid–CoA ester, which then undergoes enzymatic "thiolysis" by a molecule of CoA-SH to form a molecule of acetyl-CoA (which may then form either acetoacetate or citrate) plus a fatty acid–CoA ester containing two less carbon atoms than the original. Each of these reversible reac-

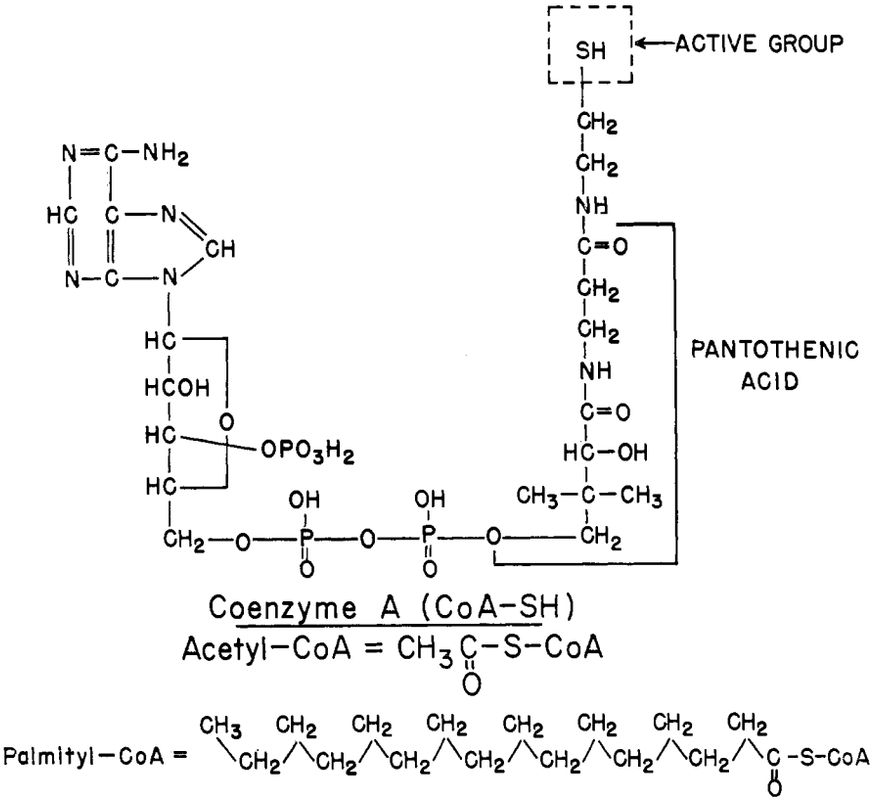
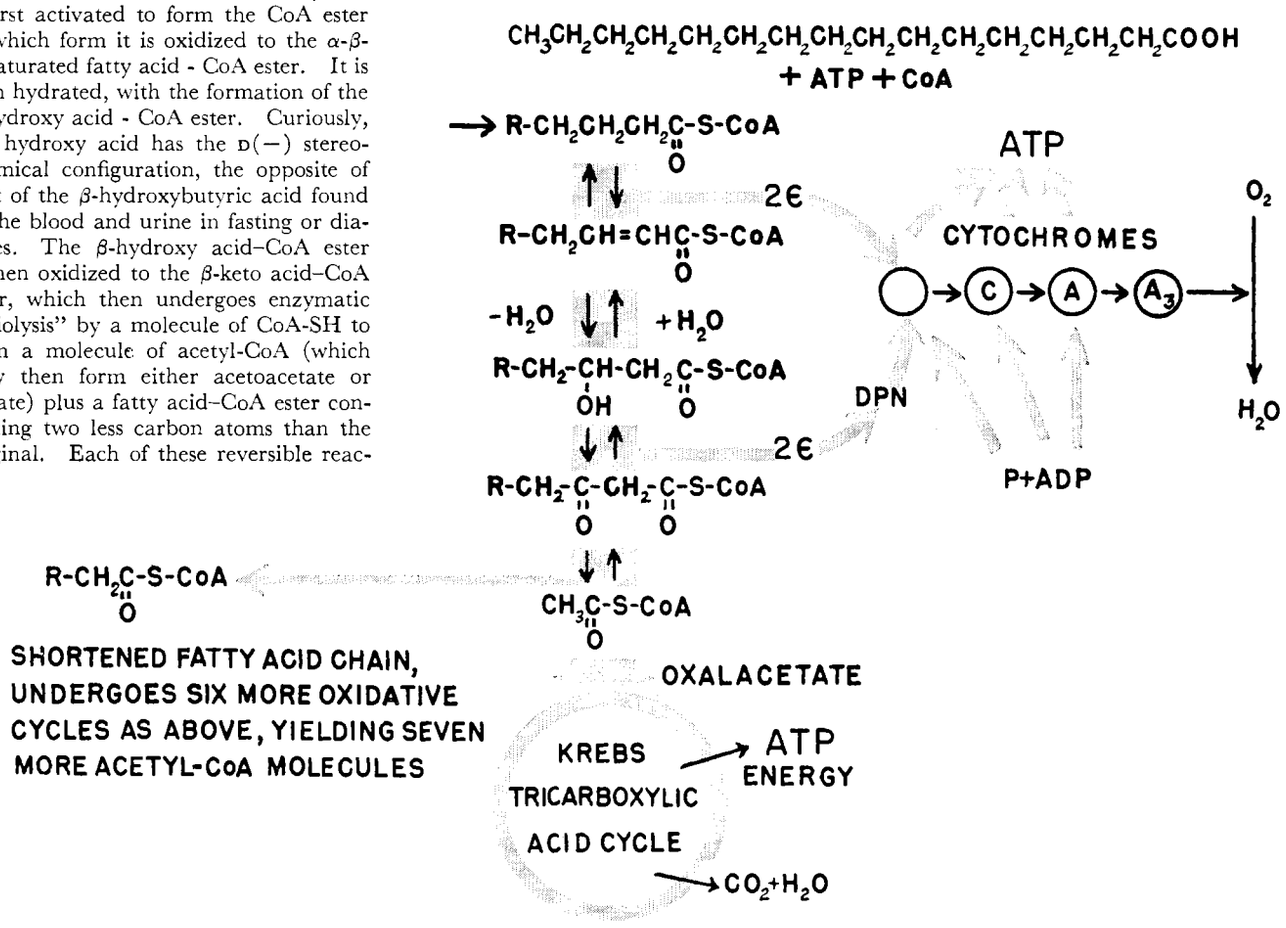


Figure 4

Figure 5. "Flow-sheet" for fatty acid oxidation



PATHWAY OF CONVERSION OF GLUCOSE INTO FAT

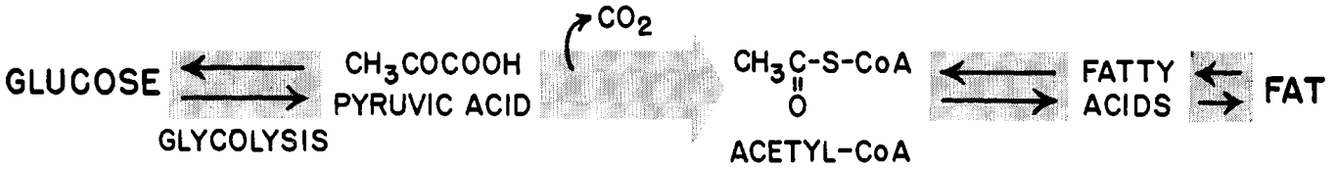


Figure 6

tions is catalyzed by a specific enzyme. The whole cycle then repeats on the shortened fatty acid–CoA complex. Ultimately the original fatty acid chain is completely degraded to acetyl-CoA fragments. From palmitic acid (C_{16}), for instance, eight acetyl-CoA units are formed by seven successive cycles through the enzymatic mill diagrammed in Figure 5.

It will be recalled that animal tissues not only oxidize fatty acids, a process which yields large amounts of energy, but they are also capable of synthesizing fatty acids at a high rate from other foodstuffs, such as carbohydrate and protein. This process of fat synthesis is a very active one, physiologically speaking, because of the unique role of fat as a storage fuel. The question arises: by what mechanism is carbohydrate converted into fat? Since all the oxidative reactions leading to degradation of fatty acids are reversible, it has appeared probable that the same enzymes catalyze the synthesis of fatty acids from acetyl-CoA, as well as the oxidation. Isotopically labeled acetate, when injected into an animal, is converted into labeled long chain fatty acids in a manner consistent with this hypothesis.

It has been known for some time that glucose is converted into pyruvic acid during the course of glycolysis in aerobic cells and it has been suspected that pyruvic acid, a three-carbon compound, is first converted into "active acetate" and carbon dioxide prior to entry into the tricarboxylic acid cycle. Very recent work in the laboratories of Ochoa and Gunsalus has definitely established this as a fact. The reaction may be written:

(4) $CH_3COCOOH + CoA + DPN \rightarrow$
(pyruvic acid)
$CH_3C\text{-}S\text{-}CoA + CO_2 + DPNH$
O
(acetyl-CoA)

The enzyme catalyzing this reaction, pyruvic dehydrogenase, has a complex prosthetic or active group containing both thiamine (vitamin B_1) and a new vitamin whose existence was not suspected until this particular enzymatic reaction was studied intensively, namely, thioctic acid. Also called Lipoic Acid. This complex reaction is therefore catalyzed by an enzyme system which contains as vital functional parts four different vitamins [pantothenic acid (in CoA), nicotinamide (in diphosphopyridine nucleotide, DPN) thiamine, and thioctic acid], and it serves as the linking reaction by which glucose can be converted into fat for storage. The metabolic channels are shown in the diagram in Figure 6.

Clearly, acetyl-CoA is at the center of this whole story and it is now known that this vitamin-containing complex of acetic acid is the basic metabolic building block involved in fat, carbohydrate, and amino acid metabolism. Furthermore, it also appears to be a basic unit in the metabolism of steroids and porphyrins (Figure 7). It thus appears to be perhaps the most fundamental intermediary metabolite in all aspects of cellular metabolic activity.

It may also be pointed out, in closing, that the intermediary metabolism of the cell is a highly interwoven affair. When we speak of the role of fat in nutrition, we must keep in mind the complex relationships between fat metabolism and that of protein and carbohydrate. We must also keep in mind that in the metabolism of fat many other nutritional factors are involved; at least six different vitamins are now known to be concerned in the metabolism of fat. Obviously, the science of nutrition must depend heavily on the efforts of investigators in the areas of intermediary metabolism and enzyme chemistry in order to have a rational scientific basis for establishing the nature of an "adequate diet." The recent advances described in this article illustrate this approach.

Figure 7