THE METABOLIC PRODUCTION OF CARBON DIOXIDE [1]

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ALL organic compounds when burned or combusted to completion yield carbon dioxide as one of the final products. Animal systems without exception contain the biochemical apparatus for burning organic compounds by molecular oxygen and thus massive CO_2 production is the hallmark of the animal body. We shall address ourselves in this review to the following questions: (1) which organic compounds can be combusted to CO_2; (2) how are these compounds formed; and (3) how does the combustion process provide energy for the needs of the body.

WHICH ORGANIC SUBSTANCES ARE COMBUSTED TO CARBON DIOXIDE?

The three main classes of organic materials in the animal body, viz., proteins, fats and carbohydrates, are all ultimately combustible. That is not to say that proteins, fats and carbohydrates are combustible as such. An elaborate preliminary preparatory process is mandatory before the stage is set for the actual combustion. For example, proteins must first be hydrolyzed to peptides; in turn the peptides must be hydrolyzed to amino acids; these in turn must be degraded to particular keto acids and only these can funnel into the cellular furnace. The nature of the preliminary manipulation to which the complex molecules have to be subjected will vary from one class to another and quite separate biochemical mechanisms are involved in the degradation of each of the three main classes of organic materials. The unifying and simplifying fact is that proteins, carbohydrates and fats ultimately give rise to much the same small molecules and thus the same cellular furnace can be used to burn the fragmentation or degradation products of each of the three classes of foodstuffs.

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HOW PROTEINS, LIPIDS AND CARBOHYDRATES ARE PREPARED FOR COMBUSTION

In essence, the preparative process is simply a matter of breaking the bonds which hold together the component molecules of the large macromolecules. Proteins can be looked upon as large molecular weight compounds composed of as many as several hundred amino acids. In the protein the component amino acids are linked one to the other by means of

peptide bonds $\begin{pmatrix} & O & H \\ & \| & | \\ -C-&N- \end{pmatrix}$ In the gut there

are specific enzymes which hydrolyze these peptide bonds, and as a result of this proteolytic action the protein is reduced to the large number of component amino acids. Not all of the amino acids can be directly transformed into derivative molecules that are capable of entering the cellular furnace. However, almost without exception, all amino

(a) $\overset{O}{\underset{\|}{H_3C-C}}-COOH$ (f) $H_2C-COOH$
                                                     $|$
                                                    $H_2C-COOH$

(b) $H_3C-COOH$

(c) $H_2C-COOH$ (g) $HC-COOH$
      $|$ $\|$
     $HC-COOH$ $HOOC-CH$
      $|$
     $H_2C-COOH$
      $|$
       H

(d) $HOC-COOH$ (h) $HOC-COOH$
      $|$ $|$
     $HC-COOH$ $H_2C-COOH$
      $|$
     $H_2C-COOH$

(e) $O=C-COOH$ (i) $O=C-COOH$
      $|$ $|$
     HCH $H_2C-COOH$
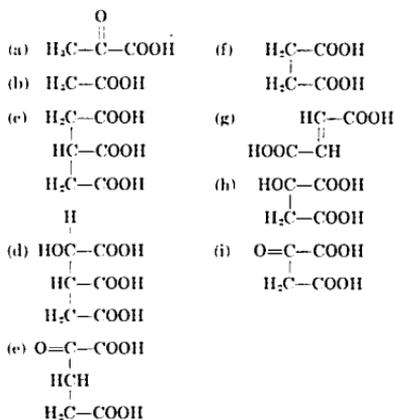 $|$
     $H_2C-COOH$

FIG. 1. The structural formulas of compounds capable of being directly combusted by the cell: (a) pyruvic acid, (b) acetic acid, (c) citric acid, (d) isocitric acid, (e) α-ketoglutaric acid, (f) succinic acid, (g) fumaric acid, (h) malic acid and (i) oxaloacetic acid. (see figure 3, The Citric Acid Cycle.)

TABLE 1

FORMATION OF CITRIC ACID CYCLE INTER-
MEDIATES AND CO_2 FROM AMINO ACIDS

Amino Acid	Key Degradation Product
alanine ⎫	
serine ⎬ → pyruvate	
cysteine ⎪	
methionine ⎭	
tyrosine ⎫ → fumarate + acetyl CoA	
phenylalanine ⎭	
glutamic acid ⎫	
proline ⎬ → α-ketoglutarate	
arginine ⎪	
histidine ⎭	
lysine →	α-ketoglutarate + CO_2
leucine →	acetyl CoA + CO_2
isoleucine →	*propionyl CoA + acetyl CoA + CO_2
threonine →	* propionyl CoA + CO_2
valine →	*propionyl CoA + CO_2
aspartic acid →	oxaloacetate
glycine →	CO_2

* Propionyl CoA is converted to a citric acid
cycle intermediate, succinic acid.

acids can be manipulated chemically in such a
way that they will give rise, in part, if not in
whole, to some molecule which is directly
combustible. The particular molecules which
are capable of direct combustion are pyruvic
acid, acetic acid, citric acid, isocitric acid,
α-ketoglutaric acid, succinic acid, fumaric acid,
malic acid and oxaloacetic acid (structural
formulas, fig. 1). As shown in table I, it is
possible to convert each of 16 amino acids
into at least one of the nine compounds cap-
able of direct combustion.

Lipids are, in essence, esters of polyalcohols,
such as glycerol or inositol. Both fatty acids
and substituted phosphoric acids, such as
choline phosphate and ethanolamine phosphate
(formulas, fig. 2) form the ester linkages with
the polyalcohols. The preparation of lipid
for ultimate combustion involves essentially
the hydrolysis of the ester bonds which hold
the molecule together. After hydrolysis by a
group of special enzymes known as esterases,
the lipid molecule is reduced to the individual
chemical building stones, namely, the free
fatty acids, glycerol phosphoric acid and the
nitrogenous bases. Of these, only the fatty
acids are of any practical importance as far
as combustion is concerned, although it is

also possible to convert the polyalcohols and
the nitrogenous bases to combustible molecules
The fatty acids can be oxidized to acetic acid
which, as mentioned above, is one of the
organic fuels for the cellular furnace.

The carbohydrates are in essence polymers
of sugar molecules held together by glycosidic
linkages. A whole group of special enzymes
are available which can hydrolyze polysac-
charides like starch or glycogen into disac-
charides and eventually into monosaccharides
In turn, the monosaccharides can now be de-
graded ultimately to pyruvic acid in a com
plex sequence of reactions collectively referred
to as glycolysis.[2] Pyruvic acid is then com-
busted to CO_2 and water in the cellular
furnace.[3]

THE MECHANISMS OF THE COMBUSTION PROCESS

There are three complex processes of a
cyclical nature which largely define the com-
bustion of organic molecules in the animal
body. These are, respectively, glycolysis,[2] the
citric acid cycle[3] and the pentose cycle.[4]

In glycolysis, sugar in the form of glucose is
cleaved into two molecules of lactic acid.

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH \qquad (1)$$
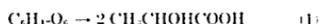
No CO_2 is formed in this sequence but the
product of the cleavage is a first cousin to
pyruvic acid which is one of the ultimate
fuels. Thus glycolysis is a device for pre-
paring the sugar molecule for combustion to
CO_2 and H_2O. However, in this particular
preparatory process, energy is harnessed even
though true combustion involving molecular
oxygen has not intervened.

The citric acid cycle is the key mechanism
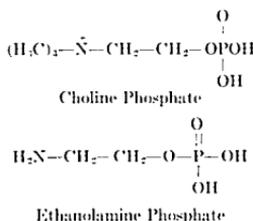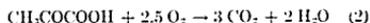for cellular combustion. In this cycle pyruvic

$$(H_3C)_3-\overset{+}{N}-CH_2-CH_2-O\overset{O}{\underset{OH}{\overset{|}{P}}}OH$$

Choline Phosphate

$$H_2N-CH_2-CH_2-O-\overset{O}{\underset{OH}{\overset{||}{P}}}-OH$$

Ethanolamine Phosphate

FIG. 2. Structural formulae of: (a) choline
phosphate and (b) ethanolamine phosphate.

acid is combusted to CO_2 and H_2O in a series of 5 oxidative steps:

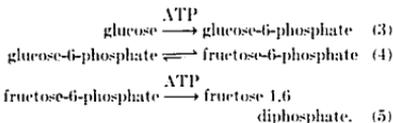$$CH_3COCOOH + 2.5\ O_2 \rightarrow 3\ CO_2 + 2\ H_2O \quad (2)$$

This is also the major system in animal tissues for trapping chemical energy in a utilizable form.

The pentose cycle is a cyclical sequence of reactions which, in effect, can oxidize glucose to CO_2 and H_2O. No energy is harnessed in the process. However, the pentose cycle rarely, if at all, operates like the citric cycle. It would be more accurate to say that the pentose cycle is yet another device for converting glucose to intermediates which are capable of entering the citric acid cycle. There are other intermediates formed in the pentose cycle which are not relevant to the combustion process and much of the meaning of the cycle has to be found in these additional functions.
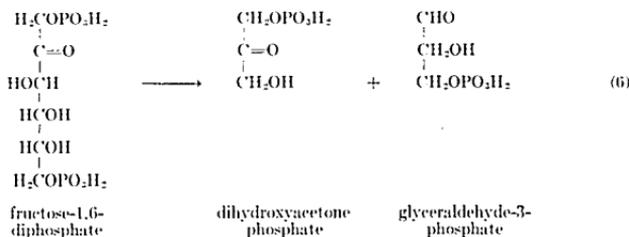
At this point it may be desirable to consider in more detail the three major cycles referred

to above. In this review the primary emphasis will be on exposing the chemical strategy, while the actual chemical changes will take a place of secondary importance.

Glycolysis. The most significant chemical event in glycolysis is the fragmentation of a 6-carbon sugar into two molecules, each containing 3 carbon atoms. It is not glucose as such which is fragmented but rather a phosphoric ester of an isomer of glucose, *viz.*, fructose. A specific phosphorylating agent known as adenosine triphosphate (ATP) and three separate enzymes are required for this conversion of glucose to fructose-1,6-diphosphate:

$$glucose \xrightarrow{ATP} glucose\text{-}6\text{-}phosphate \quad (3)$$

$$glucose\text{-}6\text{-}phosphate \rightleftharpoons fructose\text{-}6\text{-}phosphate \quad (4)$$

$$fructose\text{-}6\text{-}phosphate \xrightarrow{ATP} fructose\ 1,6\ diphosphate. \quad (5)$$

Fructose-1,6-diphosphate is cleaved into two similar but not identical halves:

$$
\begin{array}{ccc}
H_2COPO_3H_2 & CH_2OPO_3H_2 & CHO \\
| & | & | \\
C=O & C=O & CH_2OH \\
| & | & | \\
HOCH & \xrightarrow{\hspace{2cm}} \quad CH_2OH \quad + & CH_2OPO_3H_2 \quad (6) \\
| & & \\
HCOH & & \\
| & & \\
HCOH & & \\
| & & \\
H_2COPO_3H_2 & & \\
\text{fructose-1,6-} & \text{dihydroxyacetone} & \text{glyceraldehyde-3-} \\
\text{diphosphate} & \text{phosphate} & \text{phosphate}
\end{array}
$$

Each of the two fragmentation products (which are interconvertible) can in turn be converted to pyruvic acid in the following sequence of reactions:

$$glyceraldehyde\text{-}3\text{-}phosphate \rightleftharpoons dihydroxyacetone\ phosphate \quad (7)$$

$$glyceraldehyde\text{-}3\text{-}phosphate \xrightarrow{-2\ H} phosphoglyceric\ acid \quad (8)$$

$$phosphoglyceric\ acid \longrightarrow pyruvate + \text{"phosphate."} \quad (9)$$

When anaerobic conditions prevail the product of glycolysis is not pyruvate but its reduction product, lactate. Under aerobic conditions, which are more pertinent to the reactions considered in the present review, not lactate, but pyruvate, is at the end of the glycolytic sequence.

The Citric Acid Cycle.[3]   The strategy of

the citric cycle is readily apparent from a consideration of figure 3.

Pyruvate is oxidatively decarboxylated to acetate and CO_2:

$$CH_3COCOOH \xrightarrow{-2\ H} CH_3COOH + CO_2. \quad (10)$$

"Acetate" condenses with oxalacetate (7) to form citrate (1) which in turn is converted
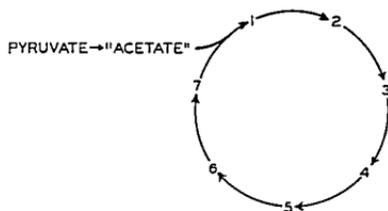
PYRUVATE→"ACETATE"

Fig. 3. The complete combustion of pyruvate by way of the citric acid cycle. The intermediates of the cycle are represented by numbers as follows: 1—citric acid, 2—isocitric acid, 3—α-ketoglutaric acid, 4—succinic acid, 5—fumaric acid, 6—malic acid, 7—oxaloacetic acid; "acetate" = acetyl-CoA. (Structural formulas are shown in fig. 1.)

back to oxalacetate in a sequence of 4 oxidative reactions (2→3, 3→4, 4→5, and 6→7). This point is more clearly shown below:
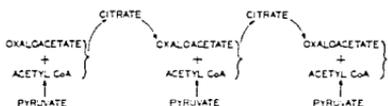

At the beginning of each cycle "acetate" is fed into the cycle via combination with oxalacetate. At the end of each cycle oxalacetate is formed. Thus each cycle achieves the complete combustion of acetate to CO_2 and water.
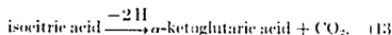
The condensation of "acetate" and oxalacetate to form citrate is a simple aldol type condensation: [5]
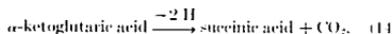

(11)
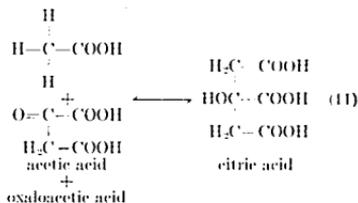
Citric acid rearranges to form isocitric acid:

(12)
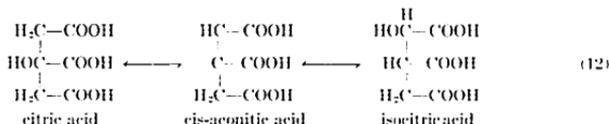
Then isocitric acid is decarboxylated to α-ketoglutaric acid:

$$\text{isocitric acid} \xrightarrow{-2H} \alpha\text{-ketoglutaric acid} + CO_2. \quad (13)$$

A second oxidative decarboxylation then takes place, this time of α-ketoglutaric acid to succinic acid:

$$\alpha\text{-ketoglutaric acid} \xrightarrow{-2H} \text{succinic acid} + CO_2. \quad (14)$$

Succinic acid is dehydrogenated to fumaric acid which in turn is hydrated to malic acid:

$$\text{Succinic acid} \xrightarrow{-2H} \text{fumaric}$$
$$\text{acid} \xrightleftharpoons{H_2O} \text{malic acid}. \quad (15)$$

Finally, malic acid is oxidized to oxaloacetic acid and that is the end of the cycle:

$$\text{malic acid} \xrightarrow{-2H} \text{oxaloacetic acid}. \quad (16)$$

In the interest of simplicity a notable omission was made. It is not acetic acid as such which enters the cycle but rather a compound of the acid with a special coenzyme known as coenzyme A (fig. 4).[6] The active group of this coenzyme is a sulfhydryl group and we may represent the coenzyme by the notation CoASH. The active form of acetate is formed by interaction with the coenzyme in the presence of a specific enzyme and ATP:[7]

$$CH_3COOH$$
$$+ CoASH \xrightarrow{ATP} CH_3COSCoA + H_2O. \quad (17)$$
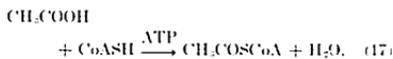
The thiol ester link in effect makes the esterified molecule much more reactive than the free acid.

Fatty acids are capable of being oxidatively and quantitatively converted to acetate and thus ultimately fatty acids can be burned to CO_2 and H_2O in the furnace of the citric cycle. Again, it is not the free fatty acid as such which is so converted, but rather the thiol ester of the fatty acid and coenzyme A and

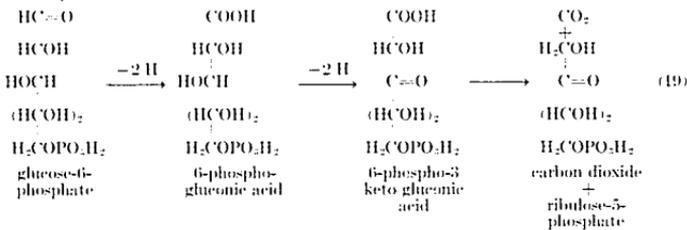he product is not free acetate but the co-
enzyme A ester thereof:

$$CH_3(CH_2)_{16}COSCoA$$
$$+ 8 H_2O \xrightarrow[8 CoASH]{-32 H} 9 CH_3COSCoA. \quad (18)$$
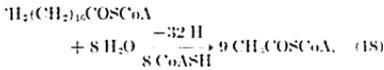
The oxidative fragmentation of the fatty acyl
CoA ester to two carbon units proceeds by way
of the well-known β-oxidation sequence.[5, 10]

Amino acids can give rise to pyruvate, oxal-
acetate, α-ketoglutarate, succinate, fumarate or
acetate—each one of which can be burned to
CO_2 and H_2O in the citric cycle (table 1). In

this way amino acids, like fatty acids, can
eventually be oxidized to CO_2 and H_2O after
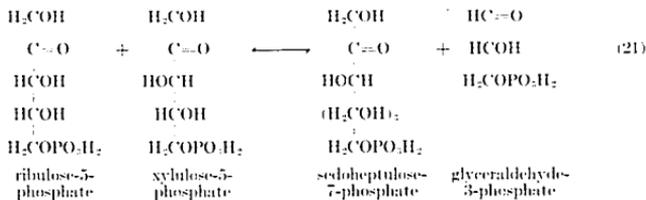appropriate preparation.

Pentose Cycle. Glucose can be converted to
pyruvic acid by a sequence of reactions other
than that shown above for glycolysis. Again
the starting point is glucose-6-phosphate which
is formed by the phosphorylation of glucose
by ATP. The aldehyde group of the sugar
phosphate is oxidized to a carboxyl and the
corresponding phosphorylated sugar acid is
then oxidatively decarboxylated to a pentose
phosphate:

$$
\begin{array}{ccccc}
HC=O & COOH & COOH & CO_2 \\
HCOH & HCOH & HCOH & H_2COH \\
HOCH \xrightarrow{-2H} & HOCH \xrightarrow{-2H} & C=O & \longrightarrow & C=O \quad (19) \\
(HCOH)_2 & (HCOH)_2 & (HCOH)_2 & (HCOH)_2 \\
H_2COPO_3H_2 & H_2COPO_3H_2 & H_2COPO_3H_2 & H_2COPO_3H_2 \\
\text{glucose-6-} & \text{6-phospho-} & \text{6-phospho-3} & \text{carbon dioxide} \\
\text{phosphate} & \text{gluconic acid} & \text{keto gluconic} & + \\
& & \text{acid} & \text{ribulose-5-} \\
& & & \text{phosphate}
\end{array}
$$

Ribulose-5-phosphate can isomerize to another pentose xylulose-5-phosphate:

$$\text{ribulose-5-phosphate} \longleftrightarrow \text{xylulose-5-phosphate.} \quad (20)$$

Then two molecules of pentosephosphate interact to form a 7-carbon sugar phosphate
(sedoheptulose-phosphate) and a 3-carbon sugar (triosephosphate):

$$
\begin{array}{ccccc}
H_2COH & H_2COH & H_2COH & HC=O \\
C=O & + & C=O & \longleftrightarrow & C=O & + & HCOH & (21) \\
HCOH & HOCH & HOCH & H_2COPO_3H_2 \\
HCOH & HCOH & (H_2COH)_2 \\
H_2COPO_3H_2 & H_2COPO_3H_2 & H_2COPO_3H_2 \\
\text{ribulose-5-} & \text{xylulose-5-} & \text{sedoheptulose-} & \text{glyceraldehyde-} \\
\text{phosphate} & \text{phosphate} & \text{7-phosphate} & \text{3-phosphate}
\end{array}
$$

Triosephosphate is convertible to pyruvic acid
according to equations 8 and 9.

The sequence of reactions from glucose to
triosephosphate via pentose phosphate is part
of the pentose cycle—an alternative to glycoly-
sis for degrading the sugar molecule. The
pentose cycle is present in many tissues but is
not of the same quantitative significance as
glycolysis.

COMBUSTION AND ENERGY PRODUCTION [11, 12]

The complete oxidation of pyruvic acid to
CO_2 and H_2O by molecular oxygen is an en-

ergy yielding process and in general metabolic
CO_2 production runs parallel with energy
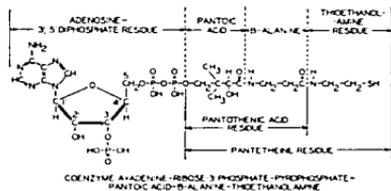generation. Part of the energy is released in

COENZYME A-ADENINE-RIBOSE-3-PHOSPHATE-PYROPHOSPHATE-
PANTOIC ACID-β-ALANINE-THIOETHANOLAMINE

FIG. 4.   The structural formula of coenzyme A.

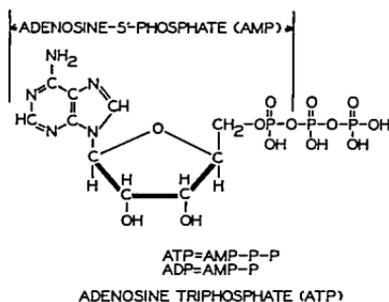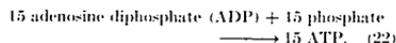ATP=AMP–P–P
ADP=AMP–P

ADENOSINE TRIPHOSPHATE (ATP)

Fig. 5.   The structural formula of adenosine triphosphate (ATP).

the form of heat and the rest in the form of the bond energy of adenosine triphosphate (fig. 5). Thus when 1 molecule of pyruvate is fully oxidized to CO_2 and H_2O by oxygen 15 molecules of ATP are synthesized:

15 adenosine diphosphate (ADP) + 15 phosphate
———→ 15 ATP.   (22)

The released energy of oxidation is harnessed to a chemical synthesis, viz., the esterification of ADP by inorganic phosphate with formation of ATP. The major source of biochemical energy is this coupling of citric cycle oxidations to synthesis of ATP.

The glycolysis of sugar to lactic acid also leads to synthesis of ATP. Thus the conversion of one molecule of sugar to two molecules of lactic acid leads to the synthesis of 2 molecules of ATP. By contrast the further oxidation of the two molecules of lactic acid to CO_2

Fig. 6.   Electron micrograph of a section of beef heart muscle showing clusters of mitochondria along the muscle fibrils.   The structures are magnified 30,300 diameters.   The photograph was prepared by K. L. Filmer and Paul Kaesburg of the University of Wisconsin.

and H_2O by way of the citric cycle leads to the synthesis of 36 molecules of ATP–18 times as much as in the glycolytic sequence.

The oxidations of the citric cycle as well as the coupled synthesis of ATP are carried out in the mitochondrion–a subcellular entity.   An electron micrograph of a cluster of mitochondria in heart muscle can be seen in figure 6. The thirty or more enzymes involved in the combustion and coupling processes are arranged and organized in a very precise pattern within the mitochondrion.[13]   The harnessing of energy involves a rather intricate sequence of events.   In each of the five oxidative steps of the citric acid cycle, electrons are transferred
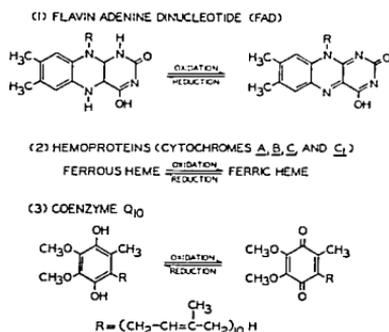

Fig. 7.   Oxidation-reduction components of the electron transfer system: (1) flavin adenine dinucleotide which contains a riboflavin moiety is the functional group of the flavoproteins; (2) the heme group of the hemoproteins (cytochromes a, b, c, and c_i) undergoes oxidation reduction; (3) coenzyme Q.[17]

from substrate to molecular oxygen through a series of catalysts capable of undergoing reduction and oxidation.   This involves a structurally linked series of enzymes collectively designated as the electron transfer chain.

The electron transfer chain contains enzymes with the following functional groups: riboflavin, heme, and a quinone known as coenzyme Q.[14]   Each of these functional groups can undergo reversible reduction and oxidation (fig. 7) as can non-heme iron and copper [15, 16] which is bound to some of the electron carrier enzymes.   Electrons are transferred from one functional group to the next in line and each such transfer involves the oxidation of the first

and the reduction of the next component. Thus electrons are passed from one catalyst to the next in a sequence of some eight or more transfers. Oxygen is the terminal electron acceptor and the final product of the reduction is water. Some three of these transfers involve a simultaneous synthesis of ATP. How this is accomplished is still unknown and is one of the pressing problems of contemporary biochemistry.

CONCLUSION

It can be seen from the foregoing discussion that carbon dioxide is mainly a by-product of the energy supplying mechanisms of the cell. While it is true that a small amount is reutilized in urea formation [18] and other carboxylation reactions,[19] the bulk of the carbon dioxide is transported by the blood to the lungs where it is expired. The mechanism of this transport will be discussed in other sections of this symposium.

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