The diagram illustrates the metabolic pathways involving the citric acid cycle and oxidative phosphorylation. Key components include:

- **Citric Acid Cycle**: Produces carbon dioxide (CO₂) and electrons (e⁻).
- **Oxidative Phosphorylation**: Generates ATP from ADP using electrons (e⁻).
- **Energy Sources**: Amino acids, sugars, glycerol, fatty acids, proteins, and polysaccharides.

The cycle begins with citric acid and CO₂, leading to the formation of Acetyl-CoA, which feeds into the pathways of various energy substrates, ultimately converging to produce ATP through oxidative phosphorylation.
Oxidative Phosphorylation

ATP is formed as electrons are transferred from NADH or UbH2 to O2 by a series of electron carriers.

OXIDATIVE PHOSPHORYLATION IN EUKARYOTES OCCURS IN MITOCHONDRIA

Mitochondria are oval-shaped organelles, typically about 2 μm in length and 0.5 μm in diameter. Eugene Kennedy and Albert Lehninger discovered that mitochondria contain the respiratory chain, the enzymes of the citric acid cycle, and the enzymes of fatty acid oxidation. Mitochondria have two membranes systems: an outer membrane and an extensive, highly folded inner membrane. The inner membrane is folded into a series of internal ridges called cristae. Hence, there are two compartments in mitochondria: the intermembrane space between the outer and inner membranes, and the matrix, which is bounded by the inner membrane. Oxidative phosphorylation takes place in the inner mitochondrial membrane, while reactions of the citric cycle and fatty acid oxidation occur in the matrix.

The outer membrane has been found to be relatively permeable to many small molecules and ions because it contains many porin molecules, (a transmembrane protein with a large pore). In contrast, the inner membrane is impermeable to nearly all ions and polar molecules. Specific protein carriers transport molecules such as ADP and long-chain fatty acids across the inner mitochondrial membrane.

In procaryotes, the respiratory chain and ATP-synthesizing complex are located in the cytoplasmic membrane, the inner of the two membranes. The outer membrane of bacteria, like that of the mitochondria, is permeable to most small metabolites because of the presence of porin.

Figure 1
The Mammalian Respiratory Chain

<table>
<thead>
<tr>
<th>Complex I</th>
<th>Fe-S</th>
<th>1 - 305 mv</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NADH-Q reductase)</td>
<td>3</td>
<td>245</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Δ</td>
<td>285 mv</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complex III</th>
<th>b-558</th>
<th>-60 mv</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b-562</td>
<td>+65 mmv</td>
</tr>
<tr>
<td>Rieske</td>
<td>2 Fe-2S</td>
<td>+280 mv</td>
</tr>
<tr>
<td>Cytochrome c1</td>
<td></td>
<td>-220 mv</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 polypeptides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>700,000 to 900,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16-33 Fe/S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 FMN</td>
</tr>
</tbody>
</table>

RESPIRATORY COMPLEXES WERE DISCOVERED AT THE INSTITUTE FOR ENZYME RESEARCH AT U. WISCONSIN—MADISON UNDER THE BRILLIANT LEADERSHIP OF PROF. DAVID GREEN
Complex IV  
(Cyt. c. oxidase)  
a  +230 mv  8 polypeptides  
Cu-1  +280 mv  160,000  
Cu-2  ?  
a3  +360 mv  

Complex II  
(Succinate-Q)  
Fe-S  1  +360 mv  4-5 polypeptides  
Reductase  2  -260 mv  6 Fe/8 S Covalently-bound  
high-potential +80 mv  140,000 FAD  

\textbf{Succinate Dehydrogenase (from Kreb's cycle)}

Components of Respiratory Chain

\textbf{a. iron-sulfur clusters}

\[
\begin{align*}
\checkmark & \quad \begin{array}{c}
\text{Fe} - +3 \\
\text{Fe} - +3
\end{array} \quad \text{Fe} - +3 \quad \text{Fe} - +3 \\
\checkmark & \quad \begin{array}{c}
\text{S} - S \\
\text{S} - S
\end{array} \\
\checkmark & \quad \begin{array}{c}
\text{S} \text{Fe} - +3 \\
\text{S} \text{Fe} - +3
\end{array} \quad +1e \quad \text{---} \quad +1e \quad \text{---} \quad +3 \text{Fe} - +3 \\
\checkmark & \quad \begin{array}{c}
\text{S} \text{Fe} - +3 \\
\text{S} \text{Fe} - +3
\end{array} \quad \text{S} - S
\end{align*}
\]

"acid labile sulfide"

\[
\begin{align*}
\checkmark & \quad \begin{array}{c}
\text{S} \text{Fe} - +3 \\
\text{S} \text{Fe} - +3
\end{array} \quad +1e \quad \text{---} \quad +1e \quad \text{---} \quad +3 \text{Fe} - +3 \\
\checkmark & \quad \begin{array}{c}
\text{S} \text{Fe} - +3 \\
\text{S} \text{Fe} - +3
\end{array} \quad \text{S} - S
\end{align*}
\]

\textbf{b. FMN or FAD}

Figure 2
c. Ubiquinone

\[
\text{CH}_3\text{O} R \text{CH}_3 + 2 H^+ + 2 e^- \rightarrow \text{CH}_3\text{O} \text{CH}_3 R \text{OH}
\]

\[
R = (\text{CH}_2 = \text{CH} = \text{CH} = \text{CH}_2)_n H
\]

Figure 3

Figure 4
ASSIGNED WAVELENGTHS FOR
THE FERROUS ALPHA BAND OF
VARIOUS RESPIRATORY CHAIN
CYTOCHROMES

Cytochrome b – Fe +2  560 nm
Cytochrome c – Fe+2  550 nm
Cytochrome c1 – Fe+2  552 nm

CYTOCHROME C-OXIDASE
  A heme-Fe+2   600 nm
  A3 heme-Fe+2  630 nm
<table>
<thead>
<tr>
<th>Complex</th>
<th>Total number of subunits</th>
<th>Number of subunits encoded by mitochondrial DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NADH dehydrogenase</td>
<td>&gt;25</td>
<td>7</td>
</tr>
<tr>
<td>2 Succinate dehydrogenase</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3 Ubiquinone:cytochrome c oxidoreductase</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>4 Cytochrome oxidase</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>5 ATP synthase</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>
The above heme structure(s) are found in hemoproteins

\( \checkmark \) Such as

(a) myoglobin \( \text{Fe}^{2+} + \text{O}_2 \rightarrow \text{Fe}^{2+}\text{-O}_2 \)

hemoglobin \( \text{Fe}^{2+} + \text{CO} \rightarrow \text{Fe}^{2+}\text{-CO} \)

(b) cytochromes \( \text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+} \)

(c) Peroxidases

Catalases \( \text{H}_2\text{O}_2 \rightarrow \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \)

P-450

Etc.

\( \checkmark \) Z can be replaced by \( \text{H}_2\text{O} \)

\( \checkmark \) inhibitor ligands

\( \text{OH}^- \)

\( \text{F}^- \)

\( \text{CN}^- \)

\( \text{CO} \)

\( \text{NO} \)

\( \text{H}_2\text{S} \)

The only cytochrome which has a replaceable Z is the a3-Fe+2 of cytochrome oxidase.

---

**Figure 5**

very intense colour

molar extinction coefficient \( \sim 150,000 \)

\( \checkmark \) \( \alpha \) for

a-type Fe+2 \( 560-650 \text{ nm} \)

b-type Fe+2 \( 560-570 \text{ nm} \)

c-type Fe+2 \( 548-555 \text{ nm} \)
ZYKLON B

PRODUCED BY TWO GERMAN FIRMS: TESCH AND DEGESCH

PRODUCED 2.75 TONS PER MONTH; USED IN DEATH CAMPS AT AUSCHWITZ THEN IN NAZI-OCCUPIED POLAND

SOLID CRYSTALS WHEN EXPOSED TO AIR TURNS INTO HCN GAS; REACTS WITH HUMAN HEMOGLOBIN-DEATH WITHIN MINUTES

DIRECTORS AFTER THE WAR CLAIMED THEY SOLD THEIR PRODUCTS FOR FUMICATION.

TWO TESCH DIRECTORS WERE SENTENCED TO DEATH AND HANGED IN 1946
Properties of mitochondrial resp. chain

(a) Tightly coupled
So NADH = 3ATP P/O=3
succinate = 2ATP P/O=2
ascorbate = 1ATP P/O=1

(b) Ageing or uncoupler causes loss of tight coupling:
uncoupler such as Dinitrophenol causes loss of ATP Formation
but stimulation of oxid. of NADH and other substrates. Why?

Explanations are found in nature of ATPase and in theories
of oxid. phosph.

(c) OSCP + OLIGOMERIN

STOPS OXID. PHOSPH

ATPase all cold-labile and have similar molecular weights
regardless of source: mammals, plants, bacterium. In
the presence of cold or HClO4, ATPase dissociates into
subunits and is inactive.

---

Pathway of Electron Transfer

\[ E = \text{NADH UB REDUCTASE} \xleftarrow{\text{Fe}^3} \xrightarrow{\text{Fe}^3} \text{ATPase} \xleftarrow{\text{ Site II (1 ATP)}} \text{ Site I (1 ATP)} \]

\[ \begin{align*}
\text{NADH} & \xrightarrow{-0.32 \text{ volts}} \text{NAD}^+ \\
\text{Fumarate} & \xrightarrow{0 \text{ volts}} \text{Succinate}
\end{align*} \]

\[ \text{Malonate inhibits Succinate.DH} \]

\[ \text{Antimycin} \]
Pathway of Electron Transfer

SITE II (1 ATP) → ATPase → SITE III (1 ATP)

2Cyt.b-Fe³⁺ 2Cyt.c₁-Fe⁺² 2 R-Fe³⁺ Fe⁺³ 2Cyt.c-Fe⁺²
2Cyt.b-Fe⁺² 2Cyt.c₁-Fe⁺³ 2 R-Fe⁺² Fe⁺³ 2Cyt.c-Fe⁺³⁺

V → 0.1V  V → 0.2V  V → 0.3V  V → 0.4V

(ARTIFICIAL ELECTRON DONOR)

E₁ a₃-Fe⁺³ E₁ a₃-Fe⁺²
O₂ Cu⁺₂ Cu⁺₁

H₂O 4H⁺ O₂

F⁻ CN⁻ CO NO H₂S

Figure 7
\[
\text{NADH} \xrightarrow{\text{O}_4} \text{NAD}^+ \rightarrow \text{Cyt b-Fe}^{+3} \rightarrow \text{Cyt c-Fe}^{+3} \rightarrow R \rightarrow \text{Cyt c-Fe}^{+3} \rightarrow \text{Cyt Oxidase} \rightarrow \text{O}_2 \rightarrow \text{H}_2 \rightarrow 2\text{Cu}^{+2} \rightarrow+8\text{e}^{\text{-}} \\
\text{ Reduction of Fe}^{2+} \rightarrow \text{Cytchrome} \\
560\text{nm} \quad 552\text{nm} \quad 550\text{nm} \\
\text{Reduced} \rightarrow \text{O}_{20} \rightarrow \text{Antiperi} \\
\text{Inhibited by Antimycin} \\
\text{DGOx} \rightarrow 3\text{W} \rightarrow +1.1 \quad +2.0 \quad +3.0 \quad +1.4 \quad +8.0 \\
\text{DGOx} \rightarrow +0.3 \quad \text{potential
Components of the mitochondrial ATP-synthesizing complex

Mass

Subunits (kd)

Role

Location

---

Matrix side

F1

380 Contains catalytic site for ATP synthesis

F0

25 Contains proton channel

F1 inhibitor

10 regulates proton flow and ATP synthesis

Oligomycin-sensitivity-conferring protein (OSCP)

23

Spherical headpiece on matrix side

Transmembrane

Stalk between F0 and F1

---
but without resp. chain
NR
ADP + Pi \longrightarrow
ATPase

When ATPase is heat or trysin-treated (proteolytic effect) then ATPase catalyzes the following Rx:

ATPase - ε
ATP \longrightarrow ADP + Pi

\[\text{AH}_2 \longrightarrow A + 2H^+ + 2\varepsilon\]

\[\text{ATP} + \text{OH} \longrightarrow \text{ADP} + P_i\]

\[\text{ATPase}\]

\[H^+\]

\[\text{Inside}\]

\[\text{Outside}\]

\[\text{Figure 9}\]

\[\text{✓ THREE THEORIES OF OXIDATIVE PHOSPHORYLATION}\]
Slater 1953 U. Amsterdam proposed the chemical theory:

\[\sqrt{\text{AH}_2 + B + C \longrightarrow \text{BH}_2 + A-C\text{ high energy intermediate}}\]
uncouplers \longrightarrow A + C + heat

\[A-C + \text{ADP} + P_i \longrightarrow A + C + \text{ATP}\]

(based on Krebs) Succinyl-CoA + GDP + Pi \longrightarrow succinate + GTP)

d cycle analogy

\[\sqrt{\text{Proposes existence of A-C; but never found;}}\]
\[\sqrt{\text{requires no membrane which is also serious flaw}}\]
\[\sqrt{\text{since ox. phosph. has been demonstrated only in the presence of membrane.}}\]

Theory is no longer accepted however there are continuous reports of proposed A-C even in recent literature.

Mitchell's Chemiosmotic theory 1961
1. Protons are transferred from inside to outside (integrity of membrane is essential)
2. Electron transfer from centers of low to high redox potential are mandatorily coupled with proton transport.
3. Net result: one H+ is transferred away from F1 side (and develops membrane electrochemical potential) for each electron
   \[ \text{H}^+/\text{e} = 1 \]

Has great explanation for role of membranes + how uncouplers work (increases permeability of membrane collapsing membrane potential).

Mitchell published 1st work showing both proton translocation and H/e of 1.

However H+/e ratio has varied between 0 \(\rightarrow\) 6 and is now accepted as 4-5. This means that H+ are coming from resp. chain components, i.e. resp. chain comp. like cyt. oxidase pumps H+ in violation of Mitchell's entire membrane role.

His Theory: suggests rapid change in pH could lead to ATP synthesis shown by Jagendorf at Johns Hopkins: by suddenly changing pH found ATP synthesis in chloroplasts; later also in mitochondria.

Wikstrom: showed reconstituted cyt. oxid. vesicles could carry out proton translocation; suggests more complexity in theory than proposed by Mitchell.

Boyer - conformational theory

Change in conformation causes major effects of ATPase and in resp. chain compounds leading to ATP synthesis

Based on observation that ATPase contains

\[
\begin{align*}
3\text{ATP} \\
2\text{ADP} \\
\hline
1\text{NET ATP}
\end{align*}
\]

When e transfer occurs, conformational change causes release of NET ATP from ATPase; also changes quaternary structure of ATPase so that e dissociates.

\[
\text{supernatant} \quad \text{Add e back to particles, reincorporates to ATPase and} \\
\text{ATP} \rightarrow \text{ADP + Pi} \\
\text{ATPase + e} \\
\text{ATP \rightarrow no reaction} \\
\text{-e}
\]

----------------------------------------------------------------------------------------------------------------
The process of science: experiment with isolated chloroplasts

pH 7

pH 4

pH 4

pH 8

ATP

TAGENDORF EXPERIMENT
Conversely: ATP-(a product of ATPase) induced formation of electron transfer component occurs.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Change in E°</th>
<th>Change in ∆G°</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH DH + ATP</td>
<td>-460 mV</td>
<td>+90 mV</td>
</tr>
<tr>
<td>Iron-sulfur cluster (1)</td>
<td>-380 mV</td>
<td>-20 mV</td>
</tr>
<tr>
<td>Iron-sulfur cluster (2)</td>
<td>-20 mV</td>
<td></td>
</tr>
<tr>
<td>∆80 mV</td>
<td>∆110 mV</td>
<td></td>
</tr>
</tbody>
</table>

Summary: Each theory has positive and negative components. Truth is a composite of Mitchell & Boyer Theories & requires further research.
Current model of Chemiosmotic Theory

Mitchell's theory suggests the following:

1. Electron transport (Complexes I, II, III) transfers protons (H+) from the intermembrane space to the matrix.
2. ATP synthase uses the proton gradient to synthesize ATP from ADP and Pi.
3. The reaction $\frac{1}{2}O_2 + 2H^+ \rightarrow H_2O$ in Complex IV releases protons back into the intermembrane space, maintaining the proton gradient.

This process couples the energy from electron transport to the synthesis of ATP, driving the proton gradient across the mitochondrial membrane.
New Enzyme Structure Reveals Cell’s Rotary Engine

CAMBRIDGE U.K.—Almost all life on Earth is powered by energy from the sun. Biochemists have known for 40 years that solar energy is converted in plants and ultimately in animals to the compound adenosine 5'-triphosphatate (ATP), a cellular "fuel" that powers chemical synthesis, communication, and motion. And the key to capturing the sun's power is the enzyme ATP synthase.

"This enzyme is found in almost all forms of life," says biochemist Harvey Penefsky of the Health Sciences Center of the State University of New York at Syracuse, adding that it "catalyzes the last step in the cell's energy conservation mechanism."

ATP synthase may be significant, but it's also a puzzle. For decades biochemists have been theorizing about just how the enzyme manages to grab hold of the necessary chemical precursors and churn out ATP at the incredibly high rate needed to fuel the rapid growth of plants. Those decades of puzzlement may be ending, however, since this week researchers took a big step toward solving the mystery with the publication of the three-dimensional structure of the catalytic region of ATP synthase in Nature.

A team at the Medical Research Council's Laboratory of Molecular Biology in Cambridge, U.K., led by biochemist John Walker, used x-ray crystallography to unravel the structure which, with a molecular mass of 371,000, is the largest asymmetric molecular structure ever to be resolved in full atomic detail.

"This is a major accomplishment and a big stimulus for the field," says biochemist Alan Senior at the University of Rochester Medical Center, who studies ATP synthase in the bacterium Escherichia coli. "This structure is going to be applicable to plants, all animal, and all bacterial ATP synthase—we can assume the mechanism will be the same."

The structure provides firm evidence for a model of the workings of ATP synthase developed around 1980 by Paul Boyer, then head of the Molecular Biology Institute at the University of California, Los Angeles. The model predicts that the globular catalytic region of ATP synthase spins like a top. In the course of one revolution, its three active regions change shape three times, helping them catch the precursors of ATP, catalyze the reaction, and release the new molecule—like a rotating production line.

Boyer, now retired, is gratified to see his ideas borne out. "This is obviously a landmark paper," he says. "It's going to have a huge impact." Biochemist Roderick Capaldi of the Institute of Molecular Biology at the University of Oregon sees support in the new results for his deductions about how movement within the ATP synthase molecule influences the enzyme's activity. He says "the Walker model makes such sense...I don't think there's a possibility that it's wrong."

While only plants and specialized bacteria can capture the energy of sunlight directly through photosynthesis, thereby storing up ATP, other cell types are able to make ATP by a process called oxidative phosphorylation, which consumes fats, carbohydrates—such as glucose—and oxygen. Whatever the energy source, the process takes place at specialized membranes such as the inner membrane of the mitochondrion, the cellular organelle where energy production and storage takes place. Energy from sunlight, or oxidation of foodstuffs, creates a build-up of positively charged hydrogen ions, or protons, on one side of the membrane. The resulting difference in proton concentration on the two sides of the membrane is known as the "proton motive force," because it makes the protons "want" to move back across the membrane to even out the disparity in concentration.

ATP synthase threads through the membranes and provides the channels through which protons can flow back across the membrane under the influence of the proton motive force; it is this proton flux that drives ATP production. The enzyme molecule has three parts. The first, designated $F_0$, threads through the membrane. The second component is a short stalk that connects $F_0$ to the third element, a globular molecule known as $F_1$, that is less than 10 nanometers

SCIENCE • VOL. 265 • 26 AUGUST 1994
across. Using an electron microscope, it is possible to see the stalks with their F₁ heads like rows of lollipops lining the inner mitochondrial membrane.

The F₁ part of ATP synthase is the catalyst that creates ATP molecules by combining ADP [adenosine 5'-diphosphate] and phosphate. In some situations, such as in anaerobic bacteria, it catalyzes the opposite reaction—splitting ATP back into ADP and a phosphate. For their crystallography study, Walker's team chose to look at F₁, and a portion of the stalk, separated from the rest of the molecule. In this independent form, F₁ is called adenosine 5'-triphosphatase, or ATPase for short. They chose ATPase because alone it has the advantage of being water soluble, making it easier to crystallize; the regular array of molecules in a crystal is essential to determining structure by x-ray crystallography.

Even with this help, the search for the structure of the enzyme wasn't an easy task. "We had crystals from the very beginning, but they were small and poorly formed and diffracted x-rays very weakly," says Walker. "Over the next 7 years or so we gradually improved the quality of the crystals by investigating a very wide range of crystallization conditions."

The first problem Walker's team had to solve was obtaining enough pure protein to grow crystals. The trick here is to find a source of tissue rich in the protein, and the Cambridge team used beef hearts, because the cardiac muscle burns ATP to fuel its contractions. As a result, the cells of the heart are packed with mitochondria: The mitochondrial membranes consist of nearly 10% ATPase. From three hearts, Walker could extract up to 100 milligrams of the enzyme in 2 days—a remarkable amount by the standards of protein chemistry. "We must have prepared a total of 30 grams of enzyme over the years," he says, adding that for future experiments he hopes to grow the protein in bacteria.

The next challenge was growing perfect crystals of the enzyme that were up to 1 millimeter across—the size needed for crystallography. The tricky part at this stage was that every protein favors slightly different conditions—such as solvent and pH—for crystallization, and pinning down just the right conditions for growing the crystal is still more of an art than a science. In addition, ATPase changes shape during catalysis, and it was important to get all the molecules in the crystal to adopt the same shape. A key finding was that "heavy" water, made with the hydrogen isotope deuterium, was the best solvent, possibly because it stabilized forces between the different parts of the enzyme, helping to make flawless crystals. Added to the solvent was a mixture of ADP and a non-hydrolyzable analog of ATP, which kept all the enzyme molecules in the same shape.

From earlier biochemical studies, the researchers knew that the globular head of ATPase consists of six subunit proteins, three of each two types, known as alpha and beta subunits. Electron microscopy and low-resolution x-ray studies showed that the six subunits are arranged in alternating fashion in a ring, much like an orange with six fat segments. The beta subunits are the ones that catalyze the creation of ATP. Boyer's model proposed that these three subunits change shape to bind the precursor molecules, allow them to react chemically, and then release the newly formed ATP; his model also proposed that each of the three was always at a different stage in the process.

This model had been disputed by some researchers, such as Mario Amzel and Peter Pedersen of Johns Hopkins University in Baltimore, who said that in their low-resolution x-ray analysis all the beta subunits had the same shape—the molecule was symmetrical. But researchers are confident that Walker's structure has cleared up such doubts. "The new structure certainly does show asymmetry between the three beta chains," says bioenergeticist Stuart Ferguson of Oxford University, who says the result was "quite widely anticipated, but this really gives you detailed insight." On Walker's evidence, "the suggestions of a symmetrical arrangement are out," says Boyer. But Amzel maintains that he and Pedersen were looking at a different freeze-frame in their rat enzyme. "There are states of the molecule more symmetrical than the one presented [by Walker]."

While Walker's structure supports the idea that ATP is produced as each beta subunit undergoes a sequence of deformations, there is still the question of how this reaction is powered by the protons flowing through the F₁ part of ATP synthase. The key to this problem is the connecting stalk, and the new x-ray structure offers guidance here too, since it contains half of the gamma subunit, the first part of the stalk (although none of the remaining six stalk subunits).

The new structure shows that the gamma subunit is a slightly curved, elongated protein running through the center of the alpha/beta ring and out at the F₁ pole. The curve is crucial, because it supports a growing consensus among biochemists that the proton flux causes a shape change in the stalk, starting at the membrane end, which translates into a relative rotation between the stalk and the F₁ head. It is not known whether it is the stalk, head, or both that actually move, but researchers are fairly certain that one rotates relative to the other. "What the structure seems to show is that ATP synthesis is driven by physical movement of the gamma chain relative to the alpha and beta units," says Ferguson.

In this model, the gamma subunit is like a knife with a curved sharp edge which is pushed into the center of the orange. If the orange twists around relative to the knife, different segments come into contact with the sharp edge of the blade in turn, and this edge deforms the shape of the segment. The relative rotation of the F₁ head against the gamma stalk brings each of the beta subunits in turn against a particular part of the gamma unit, and the nature of the contact between the two deforms part of the beta subunit into a shape that makes it pick up ADP and a phosphate. Twisted further around, the beta subunit moves into a different shape which makes the two compounds react; another third of the revolution and the shape changes to eject the newly formed ATP. Capaldi already had experimental evidence that the relative movement of the stalk and the head played an important role: He found that cross-linking the alpha/beta ring to the stalk—stopping the rotation—blocked the enzyme's activity; breaking the links restored it again.

Capaldi and other researchers are looking forward to the work they can do with the new map of ATPase. "The next phase for everyone is investigating the changes in shape—we need to see how many are different states," he says. Walker is now doing just that, as well as working on crystals of the stalk and of F₁. Senior envisages that ultimately we could "learn more about energy transfer, which could be used to improve crops and fermentation," he says. Having puzzled for years over a molecule whose structure they could not see, Walker's work has given researchers new vision. Says Capaldi: "My heart beats twice as fast as it did last week—this is very exciting."

Claire O'Brien is a science writer based in Cambridge, U.K.
Figure 21.6 Direct observation of ATP-driven rotation in ATP synthase. The $\alpha_3\beta_3$ hexamer of ATP synthase is fixed to a surface, with the $\gamma$ subunit projecting upward and linked to a fluorescently labeled actin filament. The addition and subsequent hydrolysis of ATP result in the counterclockwise rotation of the $\gamma$ subunit, which can be directly seen under a fluorescence microscope.
2 CO₂ →

Cyclic acid cycle

CO₂ →

Oxidative phosphorylation

ADP → ATP

Acetyl-CoA

Amino acids

Glucose and other sugars

Fatty acids and glycerol

Proteins

Polysaccharides

Fats
Some naturally occurring fatty acids in animals are:

- Stearate
- Palmitate
- Myristate
- Laurate
- n-Decanoate
- n-Hexadecanoate
- n-Tridecanoate
- n-Tetradecanoate
- n-Pentadecanoate

Carbon atoms 2 and 3 are often referred to as a and b.

OH
\ / a
H3C-(CH2)n-C=CH2-C2
  \ // 1
  \ 2
  / 3
H

A triacylglycerol or triglyceride is the acylated form of glycerol. They are composed of three fatty acids linked to a glycerol backbone.

Fatty acids have three major physiological roles. First, they are building blocks of phospholipids and glycerophospholipids. Second, fatty acid derivatives may serve as hormones or cytokines. Third, fatty acids are important cellular messengers. Among fatty acids, stearic acid is a non-saturating fatty acid.

FATTY ACID METABOLISM
TRIACYLGLYCEROLS ARE HIGHLY CONCENTRATED ENERGY STORES

Triacylglycerols are highly concentrated stores of metabolic energy. The yield from the complete oxidation of fatty acids is about 9 kcal/g; in comparison to about 4 kcal/g for carbohydrates and proteins.

TRIACYLGLYCEROLS ARE HYDROLYZED BY LIPASES

\[
R_1 - C \quad \text{Lipases} \quad CH_2OH
\]

\[
R_2 - C - O - CH \quad CH_2O-C-R_2 \quad + 3 \text{H}_2\text{O} \quad \rightarrow \quad HO-CH_2 - \quad + \quad R_2 - C \quad + \quad 3 \text{H}^+ \quad \text{CH}_2OH
\]

Triacylglycerol  Glycerol  Fatty acids

The activity of adipose-cell lipase is regulated by hormones such as adrenalin.

Glycerol formed by lipolysis is phosphorylated and converted to dihydroxyacetone phosphate, which then isomerizes to glyceraldehyde 3-phosphate. This intermediate is found in both glycolytic and the gluconeogenic pathways. Glycerol can be converted into pyruvate or glucose in the liver.
FATTY ACIDS ARE BOUND TO COENZYME A BEFORE THEY ARE OXIDIZED

Eugene Kennedy and Albert Lehninger showed in 1949 that fatty acids are oxidized in mitochondria.

\[
\begin{align*}
\text{R-C} & + \text{ATP} + \text{HS-CoA} \rightarrow \text{R-C-S-CoA} + \text{AMP} + \text{PPi} \\
\text{Acyl CoA} & \\
\end{align*}
\]

FATTY ACID THIOKINASE OR ACYL CoA SYNTHETASE

Note: 2 high-energy bonds are required to make the acyl CoA

CARNITINE BINDS LONG-CHAIN ACTIVATED FATTY ACIDS WHICH THEN MOVE INTO THE MITOCHONDRIAL MATRIX

Fatty acids are activated on the outer mitochondrial membrane, but they are oxidized in the mitochondrial matrix.

\[
\begin{align*}
\text{R-C-S-CoA} + \text{H3C-N'-CH2-C-CH2-C} & \rightarrow \text{HS-CoA} + \text{H3C-N'-CH2-C-CH2-C} \\
\text{Acyl CoA} & + \text{Carnitine} \\
\end{align*}
\]

Acyl carnitine is then shuttled across the inner mitochondrial membrane by a translocase.
The diagram illustrates the β-oxidation pathway, showing the conversion of fatty acyl CoA to acetyl CoA and the production of ATP. The process involves the following steps:

1. **Thiolysis (4)**: The fatty acyl CoA is shortened by two carbons, forming 3-ketoacyl CoA.

2. **Hydration (2)**: 3-Ketoacyl CoA is hydrated, forming l-3-hydroxyacyl CoA.

3. **Oxidation (3)**: l-3-Hydroxyacyl CoA is oxidized to form NADH and H⁺.

4. **Oxidation (1)**: The fatty acyl CoA is oxidized by ETF:FAD and ETF:FADH₂, forming Q and acyl-CoA dehydrogenase.

The overall process consumes 12 ATP and produces 3 ATP, indicating the energy gain or loss in the pathway.
\[ \text{\( \beta \) oxidation pathway or spiral} \]

\[ \begin{align*}
\text{e.g. Palmitoyl CoA C}_{16}-\text{acyl CoA} \\
\text{RCH}_2\text{CH}_2\text{CH}_2\text{CSCoA} \\
\text{Acyl CoA} \xrightarrow{\text{Dehydrogenase}} \text{FADH}_2 \xrightarrow{\text{Electron transfer}} \text{respiratory chain} \\
\text{RCH}_2\text{C} = \text{C} - \text{CSCoA} \xrightarrow{\text{Hydratase}} \text{Enoyl CoA} \\
\text{RCH}_2\text{C} = \text{C} - \text{CSCoA} \xrightarrow{\text{Dehydrogenase}} \text{L-3-Hydroxyacyl CoA} \\
\text{RCH}_2\text{C} = \text{C} - \text{CSCoA} \xrightarrow{\text{Keto} \text{Thiolase}} \text{Acetyl CoA} \\
\text{RCH}_2\text{C} = \text{C} - \text{CSCoA} \xrightarrow{\text{Acetyl CoA}} \text{Acetyl CoA} \\
\end{align*} \]

Thus, per 2C oxidized per turn of \( \beta \)-oxidation spiral yields:
- 2 ATP (step 1)
- 3 ATP (step 3)
- 12 ATP (step 5)
- 17 ATP

On 7th cycle, the product is CH\(_2\)Ketoacyl CoA which is
- Thiolated to 2 Acetyl CoA
- \( \text{CH}_3\text{CH}_2\text{CSCoA} \xrightarrow{\text{Thiolase}} \text{CH}_3\text{C}^\circ\text{SCO}A + \text{CH}_3\text{CSCoA} \)

\[ \text{Palmitoyl CoA (C}_{16}-\text{acyl CoA}) + 7 \text{FAD} + 7 \text{NAD}^+ + 7 \text{CoASH} + 7 \text{H}_2\text{O} \]
\[ \rightarrow 8 \text{ Acetyl CoA} + 7 \text{FADH}_2 + 7 \text{NADH} + 7 \text{H}^+ \]
YIELD OF ATPs FROM C16 ACYL COA

8 ACETYL COA x 12 ATP/ACETYL COA = 96
7 FADH2 x 2 ATP = 14
7 NADH x 3 ATP = 21
\[ \text{131 ATPs} \]
\[ \text{2 ATP} \times \]
\[ \text{129 NET ATP} \]

* 2 high-energy bonds were required to activate palmitate
\[ \text{ATP} \rightarrow \text{AMP} + 2 \text{Pi} \]

\[ \text{129 ATP} \times (-12 \text{cal/ATP}) = -1548 \text{ kcal} \times \frac{100}{-2340 \text{ kcal}} = 67\% \]

COMPLETE OXIDATION OF PALMITIC ACID TO CO2 + H2O

ANOTHER EXAMPLE IS LAURATE (C14)

C14 7 ACETYL COA x 12 = 84
6 FADH2 x 2 = 12
6 NADH x 3 = 18
\[ \text{114 ATP} \]
\[ \text{2 ATP} \times \]
\[ \text{112 NET ATP} \]
Figure 17-15
Formation of ketone bodies
THE COMPLETE OXIDATION OF PALMITATE YIELDS 129 ATP

Palmitoyl CoA + 7 FAD + 7 NAD+ + 7 CoA + 7 H2O --->
8 acetyl CoA + 7 FADH2 + 7 NADH + 7 H+

FATTY ACIDS ARE SYNTHESIZED AND DEGRADED BY DIFFERENT PATHWAYS

Fatty acid synthesis is not simply a reversal of the degradative pathway. It consists of a new set of reactions.

1. Synthesis takes place in the cytosol, in contrast with degradation, which occurs in the mitochondrial matrix.

2. Intermediates in fatty acid synthesis are covalently linked to SH group, of an acyl carrier protein (ACP). Intermediates in fatty acid oxidation are bonded to coenzyme A.

3. The enzymes of fatty acid synthesis are bound in a single polypeptide chain called fatty acid synthase. The degradative enzymes are not associated.

4. The growing fatty acid chain is elongated by the sequential addition of two-carbon units from acetyl CoA. The activated donor of two-carbon units in the elongation step is malonyl-ACP. The elongation reaction is driven by the release of CO2.

5. The reductant in fatty acid synthesis is NADPH.

6. Elongation by the fatty acid synthase complex stops with the formation of palmitate (C16). Further elongation and the insertion of double bonds are performed by other enzyme systems.
AMINO ACID DEGRADATION AND THE UREA CYCLE

Amino acids in excess of those needed for the synthesis of proteins and other biomolecules cannot be stored. Surplus amino acids are used as metabolic fuel. The α-amino group is removed and the resulting carbon skeleton is converted into a major metabolic intermediate. Most of the amino groups of surplus amino acids are converted into urea, whereas their carbon skeletons are transformed into acetyl CoA, acetoacetyl CoA, pyruvate, or one of the intermediates of the citric acid cycle. Fatty acids, ketone bodies, and glucose are formed from amino acids.

α-AMINO GROUPS ARE CONVERTED INTO AMMONIUM ION BY OXIDATIVE DEAMINATION OF GLUTAMATE

The major site of amino acid degradation in mammals is the liver. The α-amino group of many amino acids is transferred to α-ketoglutarate to form glutamate, which is then oxidatively deaminated to yield NH₄⁺.

\[
\begin{align*}
\text{H} & \quad + \\
\text{H₃N-C-R} & \quad \rightarrow \quad \text{H₃N-C-CH₂-CH₂-COO-} & \quad \rightarrow \quad \text{NH₄⁺} \\
\text{COO⁻} & \\
\end{align*}
\]

Amino acid  Glutamate

Aminotransferases catalyze the transfer of an α-amino group from an α- amino acid to an α-keto acid. These enzymes also called transaminases. Aspartate aminotransferase, one of the most important of these enzymes, catalyzes the transfer of the amino group of aspartate to α-ketoglutarate.

Aspartate + α-ketoglutarate → oxaloacetate + glutamate

Alanine aminotransferase, which is also prevalent in mammalian tissue, catalyzes the transfer of the amino group of alanine to α-ketoglutarate.

Alanine + α-ketoglutarate → pyruvate + glutamate

Ammonium ion is formed from glutamate by oxidative deamination. This reaction is catalyzed by glutamate dehydrogenase, which is unusual in being able to utilize either NAD⁺ or NADP⁺.
AMINO TRANSFERASES OR TRANSAMINASE

\[
\begin{align*}
\text{NH}_3 & \quad \text{VIT B}_6 \\
\text{H-C-CO}_2^- + \text{C-CO}_2^- & \quad \text{pyridoxal phosphate} \quad \text{C-CO}_2^- + \text{H-C-CO}_2^- \\
\text{R}_1 & \quad \text{R}_2 \quad \text{R}_1 & \quad \text{R}_2
\end{align*}
\]

ALANINE AMINOTRANSFERASE

\[\text{ALANINE} + \text{D-KETOGLUTARATE} \rightarrow \text{PYRUVATE} + \text{GLUTAMATE}\]

\[
\begin{align*}
\text{NH}_3 + \text{C-CO}_2^- + \text{CH}_2 & \quad \text{C-CO}_2^- + \text{NH}_3 \\
\text{H-C-CO}_2^- + \text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_3 & \quad \text{C-CO}_2^- + \text{CH}_2 \\
\text{ALANINE} & \quad \text{D-KETOGLUTARATE} \quad \text{PYRUVATE} \quad \text{GLUTAMATE}
\end{align*}
\]

ASPARTATE AMINOTRANSFERASE

\[\text{ASPARTATE} + \text{D-KETOGLUTARATE} \rightarrow \text{OXALOACETATE} + \text{GLUTAMATE}\]

\[
\begin{align*}
\text{NH}_3 + \text{C-CO}_2^- + \text{CH}_2 & \quad \text{C-CO}_2^- + \text{NH}_3 \\
\text{H-C-CO}_2^- + \text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{OXALOACETATE} & \quad \text{ANOTHER METHOD TO GENERATE OAA}
\end{align*}
\]

\[\text{TRANSAMINASE IS A DIAGNOSTIC ENZYMATIC ASSAY FOR HEART & LIVER DISEASES (MCH CHEM LAB 1950s)}\]

Dr. Charles Dolak+
The activity of glutamate dehydrogenase is allosterically regulated. The vertebrate enzyme consists of six identical subunits. Guanosine triphosphate (GTP) and adenosine triphosphate (ATP) are allosteric inhibitors, whereas guanosine diphosphate (GDP) and adenosine diphosphate (ADP) are allosteric activators.

The sum of the reactions catalyzed by aminotransferases and glutamate dehydrogenase is

$$\alpha\text{-Amino acid} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \alpha\text{-keto acid} + \text{NH}_4^+ + \text{NADH} + \text{H}^+$$

(or NADP+)  \quad \leftarrow

$$\text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{NADH} + \text{H}^+$$

(or NADPH)

In terrestrial vertebrates, NH$_4^+$ is converted into urea, which is excreted. The synthesis of urea will be discussed shortly.

*(Fig.1)*
NH₄⁺ is converted into urea in most terrestrial vertebrates and then excreted.

Urea is synthesized by the urea cycle. This series of reaction was proposed by Hans Krebs. The urea cycle was the first cyclic metabolic pathway to be discovered. One of the nitrogen atoms of the urea synthesized by this pathway is transferred from an amino acid, aspartate. The other nitrogen atom and the carbon atom are derived from NH₄⁺ and CO₂. Ornithine is the carrier of these carbon and nitrogen atoms.

*Fig. 2*

THE UREA CYCLE IS LINKED TO THE CITRIC ACID CYCLE
The stoichiometry of urea synthesis is
\[
\text{CO}_2 + \text{NH}_4^+ + 3 \text{ ATP} + \text{aspartate} + 2 \text{ H}_2\text{O} \rightarrow \\
\text{urea} + 2 \text{ ADP} + 2 \text{ Pi} + \text{AMP} + \text{PPi} + \text{fumarate}
\]
Four -P are consumed in these reactions to synthesize one molecule of urea. The synthesis of fumarate by the urea cycle is important because it links the urea cycle and the citric acid cycle. Fumarate is hydrated to malate, which is in turn oxidized to oxaloacetate. Oxaloacetate has several possible fates: (1) transamination to aspartate; (2) conversion into glucose by the gluconeogenic pathway; (3) condensation with acetyl CoA to form citrate; or (4) conversion into pyruvate.
*(Fig.3)*

**CARBON ATOMS OF DEGRADED AMINO ACIDS EMERGE IN MAJOR METABOLIC INTERMEDIATES**

The strategy of amino acid degradation is to form major metabolic intermediates that can be converted into glucose or be oxidized by the citric acid cycle. The carbon skeletons of the diverse set of twenty amino acids are converted into only seven molecules: pyruvate, acetyl CoA, acetoacetyl CoA, α-ketoglutarate, succinyl CoA, fumarate, and oxaloacetate.

Amino acids that are degraded to acetyl CoA or acetoacetyl CoA are termed ketogenic because they give rise to ketone bodies.

In contrast, amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl CoA, fumarate, or oxaloacetate are termed glucogenic.

Of the basic set of twenty amino acids, only leucine and lysine are purely ketogenic. Isoleucine, phenylalanine, tryptophan, and tyrosine are both ketogenic and glucogenic. The other fourteen amino acids are classed as purely glucogenic.
Cobalamin (vitamin B12) was crystallized by Dorothy Hodgkin, who determined its three-dimensional structure in 1956. The corrin ring, like a porphyrin, has four pyrrole units. Two of them (rings A and D) are directly bonded to each other, whereas the others are joined by methene bridges, as in porphyrins. A cobalt atom is bonded to the four pyrrole nitrogens.

Cobalt in cobalamin can undergo changes in oxidation states (+1, +2, or +3) B12a (Co3+), is reduced to a divalent state, called B12r (Co2+). The B12r (Co2+) form is reduced to Colt.

B12a (Co3+) \(\rightarrow\) B12r (Co2+) \(\rightarrow\) B12s (Co+)

The B12s form yields the active coenzyme. Cobalamin enzymes catalyze three types of reactions: (1) intramolecular rearrangements; (2) methylations, as in the synthesis of methionine; and (3) reduction of ribonucleotides to deoxyribo nucleotides. The conversion of L-methylmalonyl CoA into succinyl CoA (an intramolecular rearrangement) and the formation of methionine by methylation of homocysteine are the only known reactions dependent on coenzyme B12 in mammals.

Pernicious anaemia - Lack of intrinsic factor
PHENYLALANINE AND TRYOSINE ARE CONVERTED TO ACETOACETATE AND FUMARATE

First enzyme is called a monooxygenase (also called a  mixed-function oxygenase) because one atom of O2 appears in the product and the other in H2O. Oxygen is used to disrupt the aromatic ring. Absence of this enzyme results in phenylketonuria (PKU)

\[
\begin{align*}
\text{Phenylalanine} & \rightarrow \text{O}_2 \rightarrow \text{Tyrosine} \\
\text{Phenylalanine hydroxylase} & \\
\end{align*}
\]

*(Fig.5)*

PKU - Lack of phenylalanine hydroxylase results in 50% loss of IQ within 2 yrs of birth. Mental hospitals! Treatment!
Phenylalanine \rightarrow hydroxylase \rightarrow Tyrosine \rightarrow p-Hydroxyphenylpyruvate \rightarrow ALKAPTON (Homogentisate) oxidase \rightarrow 4-Maleylacetoacetate \rightarrow 4-Fumarylacetoacetate \rightarrow Fumarate + Acetoacetate