BIOLOGY 12 - ENZYMES & CELLULAR METABOLISM: CHAPTER NOTES

In order for cells to maintain homeostasis, they must constantly convert chemicals from one form to another, in order to produce necessary molecules, obtain usable molecules from food, and produce energy rich molecules.

- *These constantly occurring chemical reactions are collectively known as **metabolism**. In this chapter, you will learn about the molecules that control metabolism, **ENZYMES**.*

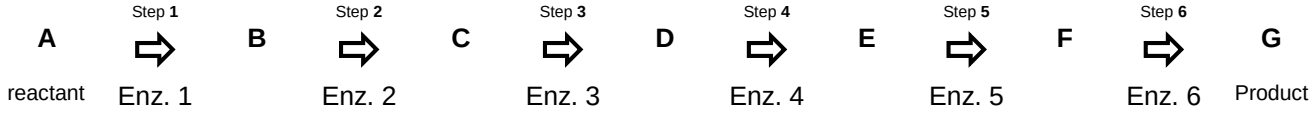
METABOLISM AND METABOLIC PATHWAYS

METABOLISM

- a term to collectively describe all the chemical reactions occurring constantly in the cell that maintain homeostasis.

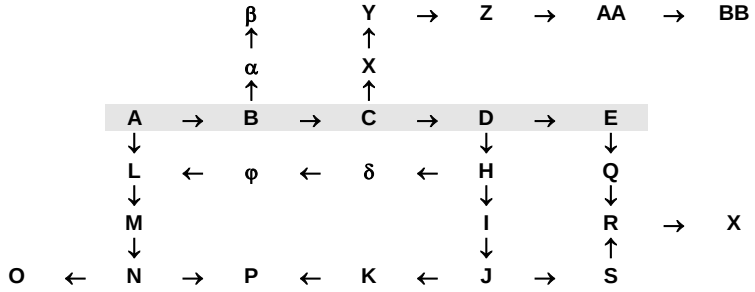
METABOLIC PATHWAYS

- the orderly step-wise series of chemical reactions from the initial **reactants** to the final **products**. One reaction leads to the next. Highly **structured**. Controlled by **enzymes**.
- each step (i.e. each chemical reaction) within the metabolic pathway requires **a SPECIFIC enzyme**.



*There are **reasons** why metabolic pathways exist:*

1. it is **not possible in biological systems to have a single reaction that could produce complex molecules** from simple reactants. (e.g. $6CO_2 + 6H_2O \Rightarrow C_6H_{12}O_6 + 6O_2$ would **never** happen in a cell in one step). **Many** intermediate steps are needed.
2. one pathway **can lead to several others** (intermediate products of one pathway can be **starting reactant** for another pathway.
3. having more than one step means that there are more places where the overall reaction can be **controlled**.

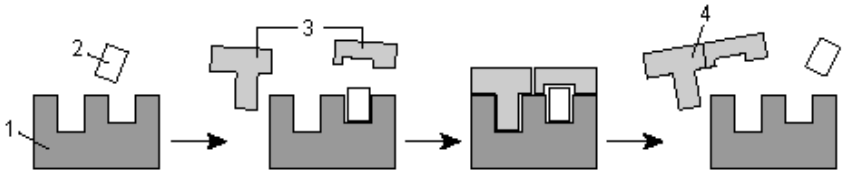


ENZYMES: Biological Catalysts

- **ENZYME** (abbr. = "E"): a **protein** that **can speed up a chemical reaction without being consumed**.
- Enzymes are the **sites** of chemical reactions, but **aren't used up in the reaction or permanently changed by the reaction.** They can, for example, hold reactant molecules together long enough for them to react.
- Enzymes are **Highly Specific**. Each enzyme speeds up only **one** reaction. Enzyme names usually end with the suffix "**ase**" (or sometimes "sin" e.g. trypsin, pepsin)
- **SUBSTRATE** ("S"): the **reactant**(s) in an enzyme's reaction.
- The equation for an enzyme-catalyzed reaction is always:

$$E + S \rightarrow ES \rightarrow E + P$$

where "ES" = **ENZYME-SUBSTRATE COMPLEX** (the chemical reaction occurs when the ES complex exists). The place where the substrates actually bind on the enzyme is known as the **ACTIVE SITE**.

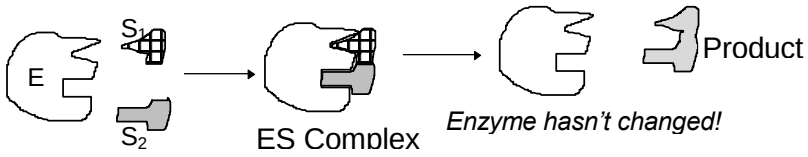


Please Label the parts of this diagram, and identify the ES complex.

How do Enzymes Work? The ***LOCK AND KEY THEORY*** vs**.** the ***Induced Fit Theory***

- Because the molecules in question are so *small* and the reaction happen so *fast*, we've never clearly seen how enzymes work. We do, however, have a *good model*. The original model, called the "**Lock and Key Theory**" has more recently been superseded by a slightly more sophisticated model called the "**Induced Fit Theory**."

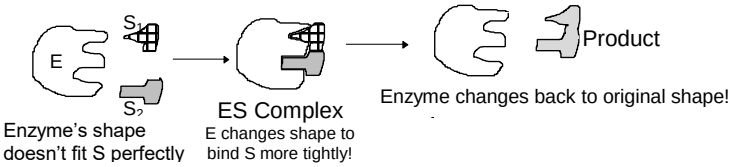
Lock and Key Theory



- *Mark in the active site on the above diagrams!*
- E and S meet during the reaction, and fit together perfectly from the very beginning, like a **lock and key**.

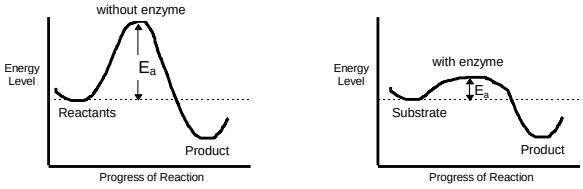
- While this model is basically correct, we now believe that instead of always remaining rigid, **the enzyme actually CHANGES SHAPE slightly** when it binds the substrates, in order to get a better tighter "grip" on the reactants. This modification of the Lock and Key theory is known as the **Induced Fit Theory**.

Induced Fit Theory



- Upon binding , the enzyme undergoes a slight **conformational change** to **more perfectly bind** the substrates.
- Then the reaction takes place, the ES complex separates, and **the enzyme re-assumes its original shape**. It is now free to catalyze another reaction.

How does an Enzyme work?



- **It LOWERS the ACTIVATION ENERGY** required for the reaction to proceed. Activation Energy is defined as **the energy that must be supplied to cause molecules to react with one another**. Enzymes do this **by bringing the substrate molecules together and holding them long enough for the reaction to take place**.

⇒ *You must be able to interpret the above graph!!*

FACTORS AFFECTING ENZYME ACTIVITY

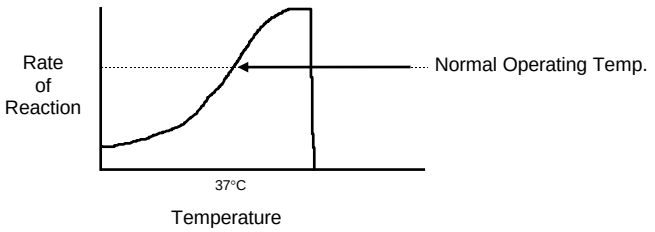
⇒ *As enzymes are proteins, they are affected by the same sorts of things that affect proteins. Since the **shape** of enzymes determines the shape of the active site, which determines their function, anything that changes the shape of an enzyme with affect the enymatic yield. Some factors are:*

1. **pH**:: most enzymes prefer pH's of 6 - 8 (some exceptions: **pepsin** in the stomach - pH ~ 2, **trypsin** in the small intestine - pH ~ 8)

- if the pH is too low **or** too high, the enzyme **DENATURES** (a denatured protein is one that **has lost its normal configuration**, and therefore its ability to form an enzyme-substrate complex).

2. **TEMPERATURE**

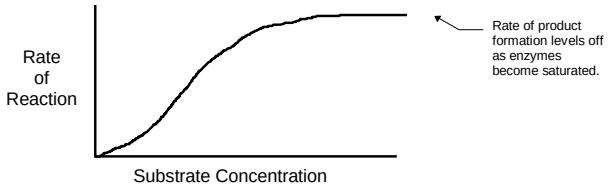
- **decreasing** temperature will **slow** rate of reaction. The lower the temperature, the lower the rate of reaction. Very low temperatures **don't** normally denature the enzyme, however.
- **increasing** the temperature slightly will, at first, **increase** the rate of reaction and product formation (as it speeds up the rate at which substrates bump into enzymes). i.e. within E's *operating range*, an *increase in Temp. will increase rate of reaction*.



- However, temperature too high (above about 45 °C) will **DENATURE** the enzyme.

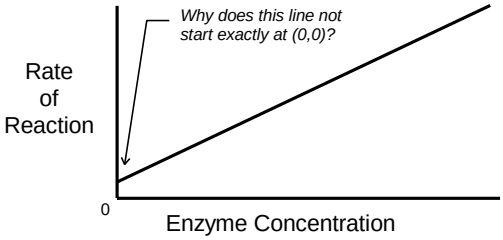
3. Concentrations of SUBSTRATES

- if the concentration (abbr. = "[ ]") of substrate increases, amount of product increases. The rate of product formation will usually increase too. However, after a certain [ ], the rate won't increase anymore, as all the enzymes are "saturated" with substrates and can't work any faster.
- if the concentration of substrate decreases, the rate of product formation will generally decrease as well.



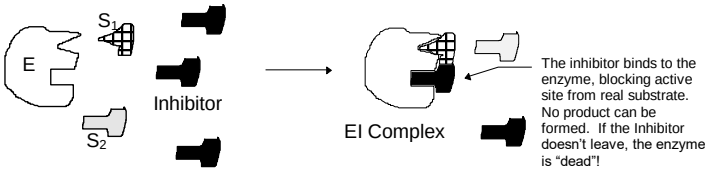
4. Concentration of ENZYME

- This is what **limits the overall rate of reaction**. Providing there is adequate substrate (and their is typically *millions* more substrate molecules than enzyme molecules**), the more enzyme you add, the more product you get**, and the **less enzyme you have, the less product you get**. In other words, if [enzyme] increases, rate of product formation increases. If amount of enzyme decreases, the rate of product formation decreases. The rate will only level off if you run out of substrate, which is usually not the case.



5. Presence of INHIBITORS

- inhibitors are molecules that **bind to the enzyme in some way to prevent or reduce the rate of substrate binding to enzyme**. There are several ways in which inhibition can work.



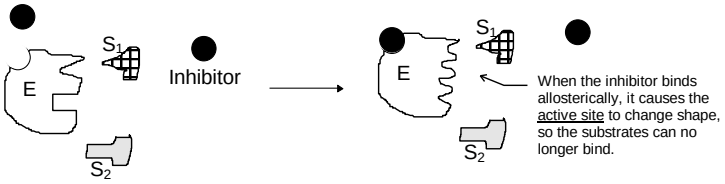
a) ***Competitive Inhibition***

- a molecule that **looks like the substrate** can **compete for space** at the active site (the place where the substrate binds to enzyme). This will **slow down** the reaction rate. The inhibitor binding to E can be **REVERSIBLE** or **IRREVERSIBLE**.

- Obviously, the more inhibitors are added, the lower the rate of reaction, and the less product is going to be made.

b) ***NON-COMPETITIVE INHIBITION***

- in this case, the inhibitor binds to **another place** on enzyme (**not** the active site). The inhibitor may look completely different from the substrate.
- When the inhibitor binds, it cause the enzyme to change shape ***at the active site*** so S cannot bind.
- binding may, as it is for competitive inhibition, be reversible or non-reversible.
- This type of inhibition is also known as "*allosteric*" inhibition.

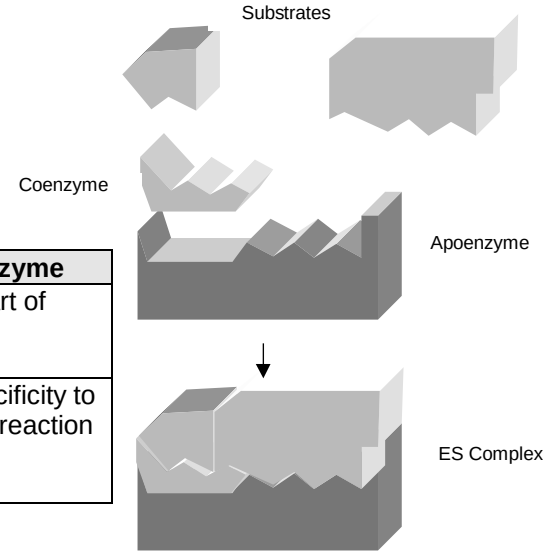


Examples of Inhibition:

- Reversible inhibition is often used as **a normal way of slowing down metabolic pathways** (e.g. an intermediate or final product may be a reversible inhibitor of another enzyme in the pathway e.g. threonine).
- Inhibitors can also be chemicals introduced into a system from the outside, and can act as medicines or poisons. e.g. **penicillin** is a medicine that kills bacteria. It works by binding **irreversibly** to the enzyme that makes bacterial cell walls.
- **HCN** (hydrogen cyanide) is a lethal **irreversible** inhibitor of enzyme action in human.
- **Lead** (Pb^{++}) and other **HEAVY METALS** (like **mercury** (Hg^{++}) and **cadmium**) are non-competitive inhibitors that cause poisoning when they bind irreversibly to enzymes and make them denature.

What are Enzymes Made of?

1) a **protein part** called an **APOENZYME** that gives it its ***specificity*** (i.e. exactly what reaction it will catalyze)
2) a **non-protein** group called a **COENZYME** which may help out the reaction by accepting or donating atoms (e.g. H^+). Label the diagram to the right.



To summarize...

Coenzyme	Apoenzyme
large non-protein molecules, many are **vitamins**. e.g. niacin (nicotinic acid) riboflavin (vitamin B_2), folic acid, biotin (vitamin H), thiamine (vitamin B_1)	protein part of enzyme
Helps reaction - may participate in reaction by accepting or giving atoms to the reaction. e.g. **NAD cycle** NAD (nicotinamide adenine dinucleotide) is a coenzyme of many oxidation-reduction reactions.	gives specificity to particular reaction

Thyroid – gland affecting metabolism

The thyroid gland is a large gland located in the neck. It actively requires iodine to produce the hormones: **thyroxine** and triiodothyronine. These hormones increase the metabolic rate. They do not have a target organ; instead, they stimulate all cells of the body and metabolize at a faster fate. More glucose is broken down and more energy is utilized. People with problems with their thyroid typically experience a lack of energy (lethargy).

OXIDATION AND REDUCTION

Oxidation

- **oxidation is the removal of hydrogen atoms**
- there are other definitions (i.e. *the removal of electrons* or *the addition of oxygen*, but we won't worry about these).
- the oxidation of a compound is accompanied by the **release of energy**. i.e. when you oxidize something, you get energy

Reduction

- reduction is the opposite of oxidation
- our definition: the addition of hydrogen atoms
- reduction is an **energy-requiring reaction.** i.e. to reduce something, you need to add energy to make the reaction go.

Lets look at a specific example!

- e.g. when you **burn something**, like methane gas (CH_4), you are performing an oxidation reaction:

$$\mathbf{CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O + ENERGY}$$

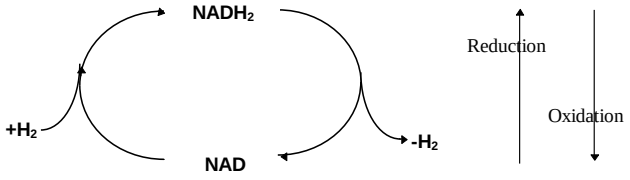
in this example...

- CH_4 has been oxidized to CO_2
- energy has been released.

This same reaction could also be made to go in the opposite direction:

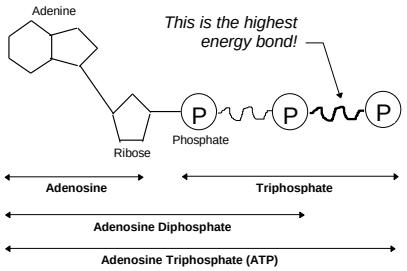
$$\mathbf{CO_2 + 2H_2O + ENERGY \rightarrow CH_4 + 2O_2}$$

- this is a **reduction reaction**...
  - CO_2 has been reduced to CH_4
  - energy has had to have been added to the system to make it work.
- usually, when one molecule is oxidized (loses H atoms), another molecule is reduced (gains those same H atoms). These combination of a reduction and an oxidation reaction are called **REDOX reactions**.
- A good example of this is the NAD cycle:



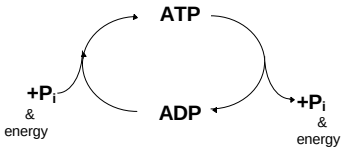
ATP - The Molecule of Energy

- cells use **Adenosine Triphosphate** when they require energy. It is the **currency** of biological energy transfers (just like the dollar is the currency of money transfers). ATP is used for all reactions requiring energy, like synthesis, muscle contraction, active transport, etc.)
- ATP is a **nucleotide** composed of the base **adenine** and the sugar **ribose**, plus three **phosphate groups**.



- ATP has **two high-energy P - P bonds** (phosphate bonds). When they **break**, **lots of energy is released**.
- ATP breaks down to **ADP** (adenosine diphosphate) and inorganic phosphate (P or "P_i") when it releases energy.
- If energy is added to the system, the reverse action will occur.

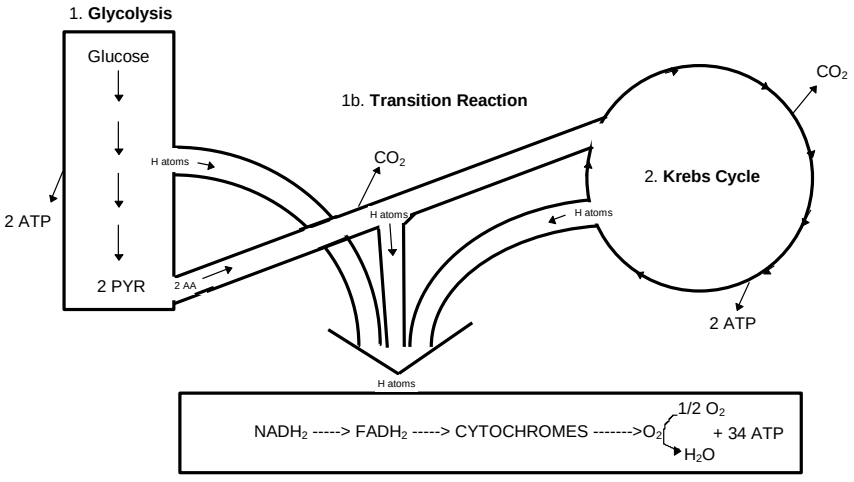
ATP → ADP + P + Energy
ADP + P + Energy → ATP



- ATP, ADP and P_i are not destroyed during energy transfers. They are constantly being formed and broken down.

AEROBIC CELLULAR RESPIRATION - this will not be covered on your Provincial Exam

- *Almost all organisms, whether they reside in the water or on land, take in oxygen and carry on aerobic cellular respiration. During this process, food molecules are oxidized and the energy released is used to form ATP molecules. The oxidation of glucose to carbon dioxide and water provides the energy for producing ATP from ADP and P_i.*
- ***Least* Specific Definition**: process of **OXIDATION/REDUCTION REACTIONS** in the **PRESENCE OF OXYGEN** that produces **ENERGY** (that is then stored in ATP) from **FOOD**.



3. **Respiratory Chain (Electron Transport Chain = Cytochrome System**

- **all plants and animals** carry on aerobic respiration (or any cells that have evolved in presence of oxygen. **Why**? ***This process releases more energy than any other.***
- This reaction is **actually a long metabolic pathway** (many steps)

Overall Reaction:

38 ADP + 38 P_i					→	***+38 ATP (ENERGY)***
$C_6H_{12}O_6$	+	$6O_2$	→	$6CO_2$	+	$6H_2O$
Food (Glucose)		oxygen		carbon dioxide		Water

(other substrates (e.g. fats) are possible)

- ***The whole process has 3 main subpathways:***

1. **GLYCOLYSIS**: *"glucose splitting"*

- each molecule of **glucose** broken into two molecules of **PYRUVIC ACID** ("**PYR**" -- sometimes also called *pyruvate*). Produces a *net total* of **2 molecules ATP**.

1b. **TRANSITION REACTION - PYR converted to active acetate (AA)**

2. **KREBS CYCLE**: produces 2 ATP and frees H atoms that will later used to produce more ATP. Occurs in the matrix of the mitochondrion.
3. **RESPIRATORY CHAIN**: *(also called the **electron transport chain** or **ETC**. Also called a "**cytochrome system"** as many molecules within it are cytochromes).* Produces up to 34 ATP per glucose by extracting the energy from the hydrogen atoms released by glycolysis, transition reaction, and Krebs cycle. Oxygen is the final acceptor of electrons and Hydrogen ions, which cause water to be produced.

Pathway	Result
Glycolysis	• **Produces 2 ATP** molecules per glucose directly • Removal of H_2 from substrates to form $2NADH_2$, which in the respiratory chain cause the formation of 6 more ATP.
Transition Reaction	• Releases $2CO_2$ per glucose • Removal of H_2 from substrates to form $2NADH_2$, which in the respiratory chain cause the formation of 6 more ATP.
Krebs Cycle	• **Produces 2 ATP** per glucose after two turns • Removal of H_2 from substrates to form 6 $NADH_2$ and 2 $FADH_2$ which in the respiratory chain cause the formation of 18 and 4 more ATP, respectively. • Releases $4CO_2$ per glucose
Respiratory Chain	• Accepts H_2 from other pathways and passes them on to O_2 producing H_2O and ATP. • **Produces 34 ATP** per glucose

Intermediates of Cellular Respiration

- The many intermediates that we encounter along the way during cellular respiration can be used, in many cases, as starting points for other reactions. For example:

Intermediate	Produced By What Process?	Can be Used to Make...
Glucose-6-Phosphate	glycolysis	glycogen
PGAL	glycolysis	glycerol, fatty acids
Active Acetate	transition reaction	fatty acids, amino acids
Pyruvic Acid	glycolysis	amino acids
oxaloacetate	Krebs cycle	amino acids
α-ketoglutarate	Krebs cycle	amino acids

- Besides glucose, other molecules can be "burned" to produce energy for cells.
- **Fats**, for example can be used: first it is broken down to glycerol and fatty acids. Glycerol can be converted to **PGAL** and enter the glycolysis pathway, and fatty acids can be converted to active acetate. *recall that fatty acids are long chains of carbons, and that AA is a two carbon compound. Therefore, conversion of fatty acids in this manner will produce **lots** of AA, which in turn will lead to the formation of lots of energy. This is why fat is a **good energy-storage molecule (fat = 9 cal/g vs. carb. = 4 cal/g).***
- **Amino acids** can also be converted (through a process called deamination) into PYR or AA and enter the cycle to be used for energy (proteins = 4 cal/g).

The following procedure was conducted to observe the effect of pH on the rate of enzyme activ

- 10 mL of a starch solution was added to each of 5 lettered test tubes.
- A different pH buffer was added to each tube resulting in the pH shown in the table below.
- An equal amount of a starch-digesting enzyme was added to each tube.
- Fresh samples were taken from **each tube every minute** and tested with IKI, an indica that turns from yellow to black when mixed with starch.

Results are recorded in the table below:

Test tube	pH of the solution	Colour of a sample when IKI was added after:			
		1 minute	2 minutes	3 minutes	4 minutes
V	5	black	black	yellow	yellow
W	6	black	yellow	yellow	yellow
X	7	black	black	yellow	yellow
Y	8	black	black	black	yellow
Z	9	black	black	black	black

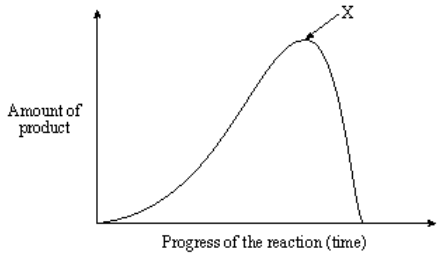
a) What do the results indicate is present in **all** the test tubes at one minute? **(1 mark)**

b) What new substance is present in test tube **X** at three minutes? **(1 mark)**

c) Which test tube has the optimal pH for the enzyme? Explain your choice. **(2 marks)**

d) After one hour, a sample from test tube **Z** still turned black. Using the lock and key model of enzyme action, explain these results. **(2 marks)**

The graph below shows the rate of product formation in an enzyme-catalyzed reaction.



The change observed at **X** could result from the addition of

A. lead.
B. a coenzyme.
C. more enzyme.
D. more substrate.