Mr. Ulrich
AP Biology

Name:.....

Enzymes Packet

Enzymes

How Enzymes Work

Enzyme Reaction Rates

Enzyme Cofactors

Enzyme Inhibitors

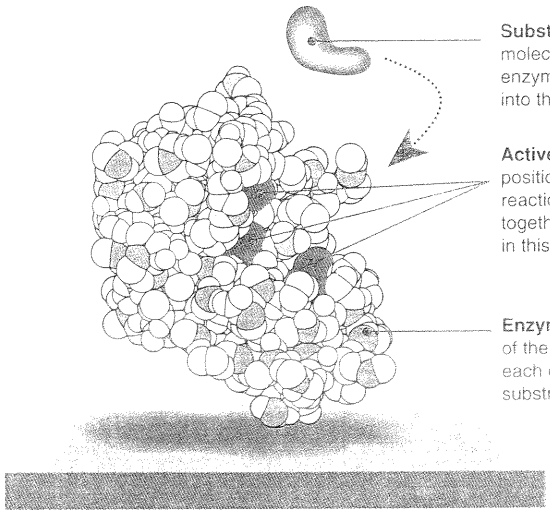
Enzymes

Most enzymes are proteins. They are capable of catalyzing (speeding up) biochemical reactions and are therefore called biological **catalysts**. Enzymes act on one or more compounds (called the **substrate**). They may break down a single substrate molecule into simpler substances, or join two or more substrate molecules together. The enzyme itself is unchanged in the reaction. Its presence merely allows the reaction to take place more rapidly. The part of the enzyme into which the substrate binds and undergoes reaction is the **active site**. It is a function of the polypeptide's complex tertiary structure.

Enzyme Structure

The model on the right illustrates the enzyme *Ribonuclease S*, which breaks up RNA molecules. It is a typical enzyme, being a globular protein and composed of up to several hundred amino acids. The darkly shaded areas are part of the **active site** and make up the **cleft**; the region into which the substrate molecule(s) are drawn.

The correct positioning of these sites is critical for the catalytic reaction to occur. The substrate (RNA in this case) is drawn into the cleft by the active sites. By doing so, it puts the substrate molecule under stress, causing the reaction to proceed more readily.

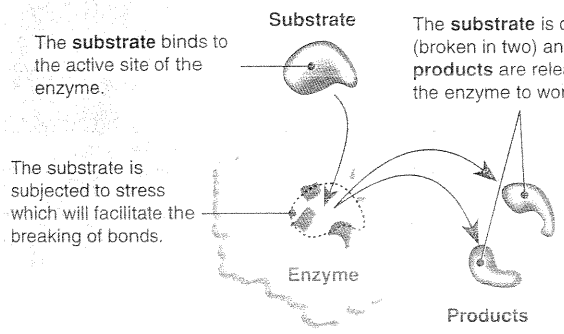


Substrate molecule: Substrate molecules are the chemicals that an enzyme acts on. They are drawn into the cleft of the enzyme.

Active site: Substrate molecule(s) are positioned in a way to promote a reaction: either joining two molecules together or splitting up a larger one (as in this case).

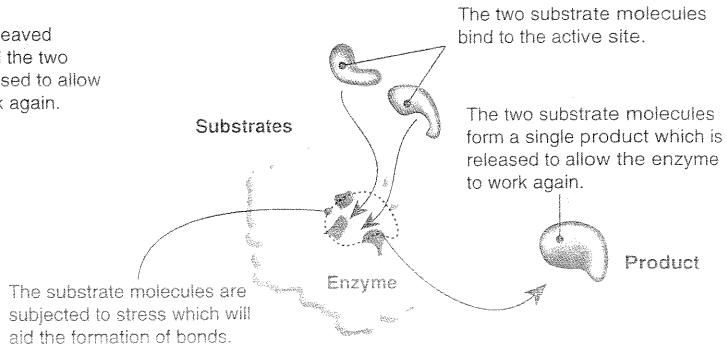
Enzyme molecule: The complexity of the active site is what makes each enzyme so specific for the substrate it acts on.

Source: After *Biochemistry*, (1981) by Lubert Stryer



Catabolic reactions

Some enzymes can cause a single substrate molecule to be drawn into the active site. Chemical bonds are broken, causing the substrate molecule to break apart to become two separate molecules. Catabolic reactions break down complex molecules into simpler ones and involve a net release of energy, so they are called exergonic. **Examples**: *hydrolysis, cellular respiration.*



Anabolic reactions

Some enzymes can cause two substrate molecules to be drawn into the active site. Chemical bonds are formed, causing the two substrate molecules to form bonds and become a single molecule. Anabolic reactions involve a net use of energy (they are endergonic) and build more complex molecules and structures from simpler ones. **Examples:** *protein synthesis, photosynthesis.*

1. Explain what is meant by the **active site** of an enzyme and relate it to the enzyme's tertiary structure:

2. What might happen to an enzyme's activity if the gene encoding its production was altered by a mutation?

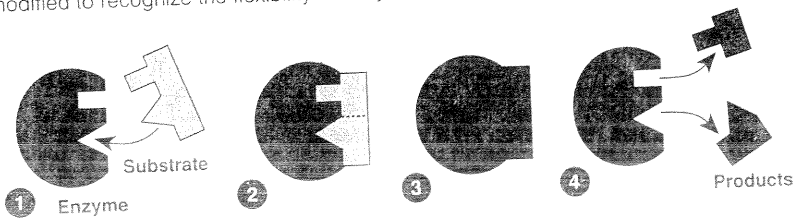
3. Distinguish between **catabolism** and **anabolism**, giving an example of each and identifying each reaction as **endergonic** or **exergonic**:

How Enzymes Work

Chemical reactions in cells are accompanied by energy changes. Any reaction, even an exergonic reaction, needs to raise the energy of the substrate to an unstable **transition state** before the reaction will proceed (below left). The amount of energy required to do this is the activation energy (E_a). Enzymes work by lowering the E_a for any given reaction. They do this by orienting the substrate, or by adding charges or otherwise inducing strain in the substrate so that bonds are destabilized and the substrate is more reactive. The current 'induced-fit' model of enzyme function is supported by studies of enzyme inhibitors, which show that enzymes are flexible and change shape when interacting with the substrate.

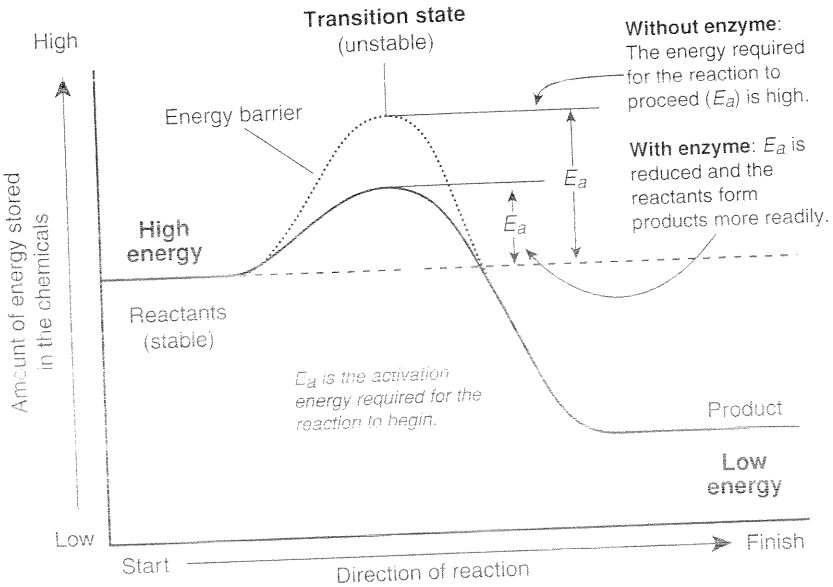
How Enzymes Work

The **lock and key** model proposed earlier last century suggested that the (perfectly fitting) substrate was simply drawn into a matching cleft on the enzyme molecule (below). This model was supported by early X-ray crystallography but has since been modified to recognize the flexibility of enzymes (the **induced fit** model, described right).



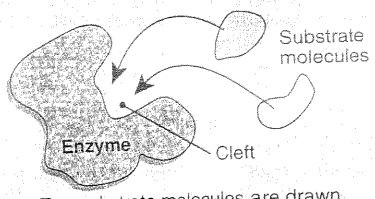
Lowering the Activation Energy

The presence of an enzyme simply makes it easier for a reaction to take place. All catalysts speed up reactions by influencing the stability of bonds in the reactants. They may also provide an alternative reaction pathway, thus lowering the activation energy (E_a) needed for a reaction to take place (see the graph below).

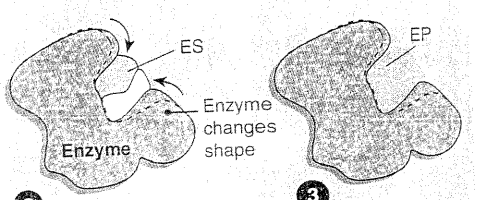


The Current Model: Induced Fit

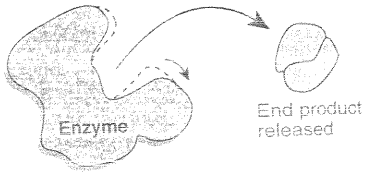
An enzyme's interaction with its substrate is best regarded as an induced fit (below). The shape of the enzyme changes when the substrate fits into the cleft. The reactants become bound to the enzyme by weak chemical bonds. This binding can weaken bonds within the reactants themselves, allowing the reaction to proceed more readily.



1 Two substrate molecules are drawn into the cleft of the enzyme.



The enzyme changes shape as the substrate molecules bind in an enzyme-substrate complex (ES). The enzyme-substrate interaction results in an intermediate enzyme product (EP) complex.



4 The end product is released and the enzyme returns to its previous shape.

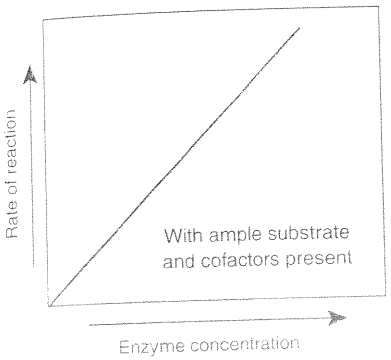
1. Explain how enzymes act as **biological catalysts**:

2. Describe the key features of the '**lock and key**' model of enzyme action and explain its deficiencies as a working model:

3. Describe the current '**induced fit**' model of enzyme action, explaining how it differs from the lock and key model:

Enzyme Reaction Rates

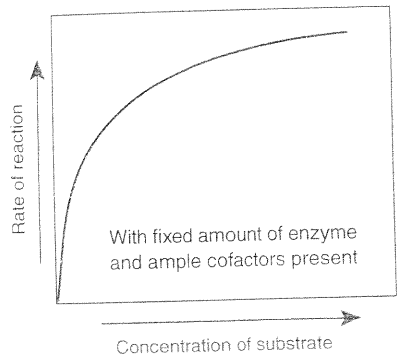
Enzymes are sensitive molecules. They often have a narrow range of conditions under which they operate properly. For most of the enzymes associated with plant and animal metabolism, there is little activity at low temperatures. As the temperature increases, so too does the enzyme activity, until the point is reached where the temperature is high enough to damage the enzyme's structure. At this point, the enzyme ceases to function: a phenomenon called **denaturation**. Extremes in acidity and alkalinity (pH) can also cause the protein structure of enzymes to denature. Poisons often work by denaturing enzymes or occupying the enzyme's active site so that it does not function. In some cases, enzymes will not function without cofactors, such as vitamins or trace elements. In the four graphs below, the *rate of reaction* or *degree of enzyme activity* is plotted against each of four factors that affect enzyme performance. Answer the questions relating to each graph:



1. Enzyme concentration

(a) Describe the change in the rate of reaction when the enzyme concentration is increased (assuming there is plenty of the substrate present):

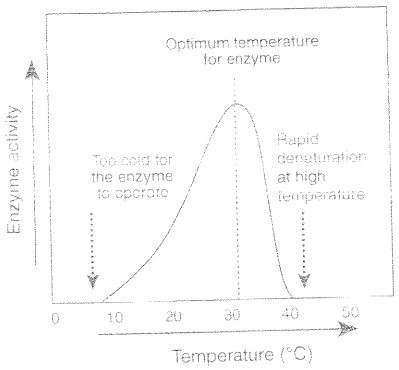
(b) Suggest how a cell may vary the amount of enzyme present in a cell:



2. Substrate concentration

(a) Describe the change in the rate of reaction when the substrate concentration is **increased** (assuming a fixed amount of enzyme and ample cofactors):

(b) Explain why the rate changes the way it does:

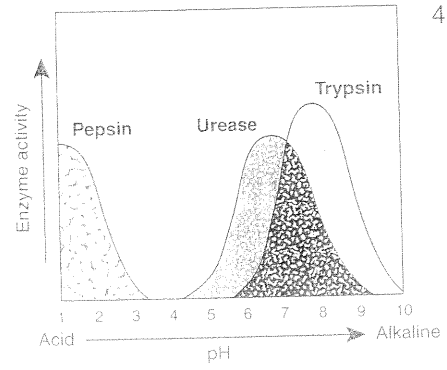


3. Temperature

Higher temperatures speed up all reactions, but few enzymes can tolerate temperatures higher than 50–60°C. The rate at which enzymes are **denatured** (change their shape and become inactive) increases with higher temperatures.

(a) Describe what is meant by an *optimum temperature* for enzyme activity:

(b) Explain why most enzymes perform poorly at low temperatures:



4. Acidity or alkalinity (pH)

Like all proteins, enzymes are **denatured** by *extremes* of **pH** (very acid or alkaline). Within these extremes, most enzymes are still influenced by pH. Each enzyme has a preferred pH range for optimum activity.

(a) State the optimum pH for each of the enzymes:

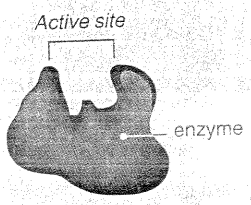
Pepsin: _____ Trypsin: _____ Urease: _____

(b) Pepsin acts on proteins in the stomach. Explain how its optimum pH is suited to its working environment:

Enzyme Cofactors

Nearly all enzymes are made of protein, although RNA has been demonstrated to have enzymatic properties. Some enzymes (e.g. pepsin) consist of only protein. Other enzymes require the addition of extra non-protein components to be functional. In these cases, the protein portion is called the **apoenzyme**, and the additional chemical component is called a **cofactor**. Neither the apoenzyme nor the cofactor has catalytic activity on its own. Cofactors may be organic molecules (e.g. vitamin C and the coenzymes in the respiratory chain) or inorganic ions (e.g. Ca^{2+}, Zn^{2+}). They also may be tightly or loosely bound to the enzyme. Permanently bound cofactors are called **prosthetic groups**, whereas temporarily attached molecules, which detach after a reaction are called **coenzymes**. Some cofactors include both an organic and a non-organic component. Examples include the heme prosthetic groups, which consist of an iron atom in the center of a porphyrin ring.

Protein-only enzymes

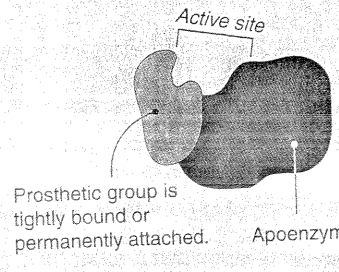


No cofactor

Functional enzyme consists of only protein
e.g. lysozyme, pepsin

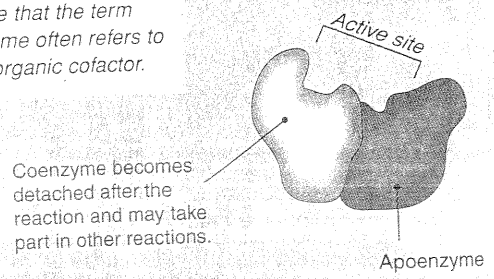
Conjugated protein enzymes

Note that the term coenzyme often refers to any organic cofactor.



Prosthetic group required

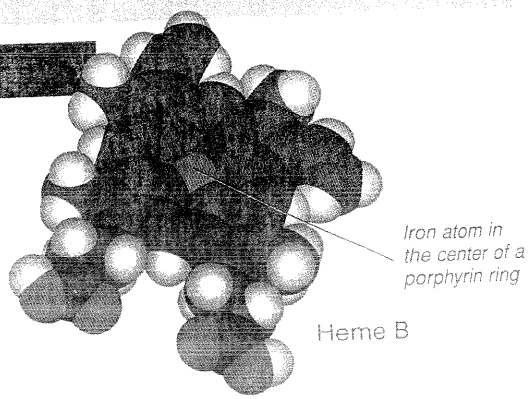
Contains apoenzyme (protein) plus a prosthetic group
e.g. flavoprotein + FAD



Coenzyme required

Contains apoenzyme (protein) plus a coenzyme (non-protein)
e.g. dehydrogenases + NAD

Enzyme cofactor	Enzyme
Cupric (copper ion)	Cytochrome oxidase
Ferrous or ferric (iron ion)	Catalase and cytochrome (via heme)
Selenium	Glutathione peroxidase
Magnesium	Glucose 6-phosphatase
Flavin	NADH dehydrogenase
Heme L (derived from heme B)	Peroxidases, e.g. thyroid peroxidase



1. Describe the general role of **cofactors** in enzyme activity: _____

2. Explain exactly how cofactors enable an enzyme's catalytic activity: _____

3. Distinguish between the apoenzyme and the cofactor: _____

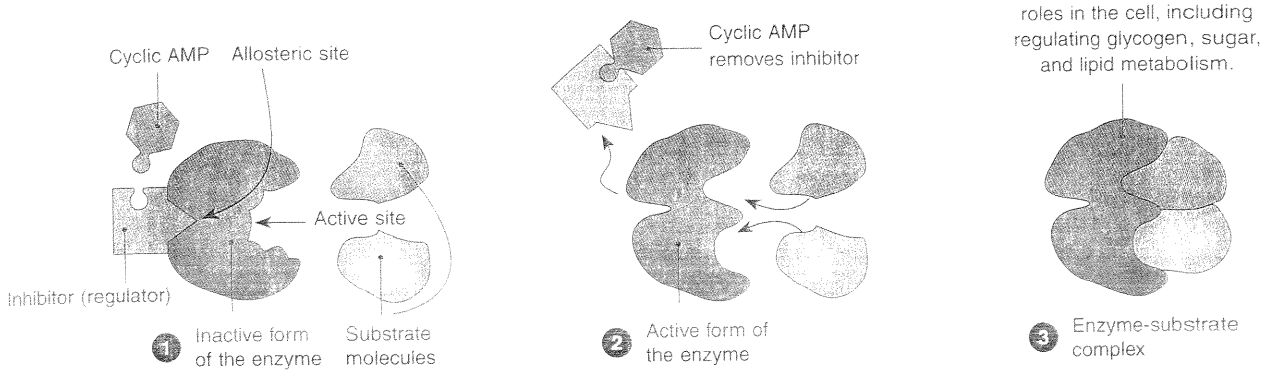
4. Identify the two broad categories of cofactors and describe an example of each: _____

5. Describe the importance of adequate vitamin and mineral intake in the diet: _____

Enzyme Inhibitors

Enzymes may be deactivated, temporarily or permanently, by chemicals called enzyme inhibitors. **Irreversible inhibitors** bind tightly to the enzyme, either at the active site or remotely from it, and are not easily displaced. **Reversible inhibitors** can be displaced from the enzyme and have a role as enzyme regulators in metabolic pathways. **Competitive inhibitors** compete directly with the substrate for the active site, and their effect can be overcome by increasing the concentration of available substrate. A **non-competitive inhibitor** does not occupy the active site, but distorts it so that the substrate and enzyme can no longer interact. Both competitive and non-competitive inhibition may be irreversible, in which case the inhibitors involved act as poisons.

Allosteric Enzyme Regulation



Allosteric regulators have a receptor site, called the **allosteric site**, on a part of the enzyme other than the active site. When a substance binds to the allosteric site, it regulates the activity of the enzyme. Often the action is inhibitory (as shown above for protein kinase A), but allosteric regulators can also switch an enzyme from its inactive to its active form. Thus, they can serve as regulators of metabolic pathways. The activity of the enzyme **protein kinase A** is regulated by the level of **cyclic AMP** in the cell. When a regulatory inhibitor protein binds reversibly to its allosteric site, the enzyme is inactive. Cyclic AMP removes the allosteric inhibitor and activates the enzyme.

Competitive Inhibition

Competitive inhibitors compete with the normal substrate for the enzyme's active site.

If a competitive inhibitor occupies the active site only temporarily then the inhibition will be reversible.

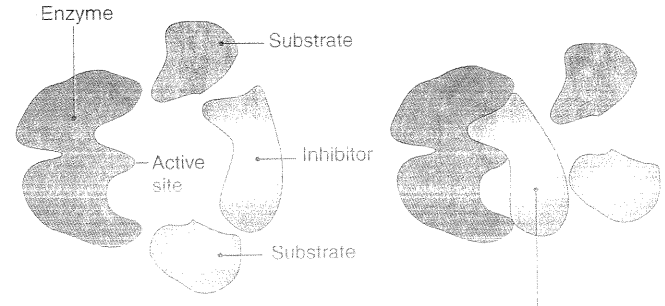
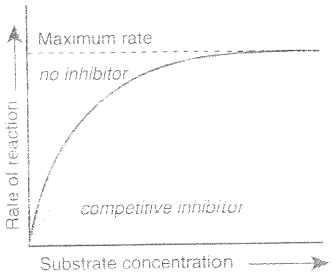


Figure 1 Effect of competitive inhibition on enzyme reaction rate at different substrate concentration



Non-competitive Inhibition

Non-competitive inhibitors bind with the enzyme at a site other than the active site. They inactivate the enzyme by altering its shape.

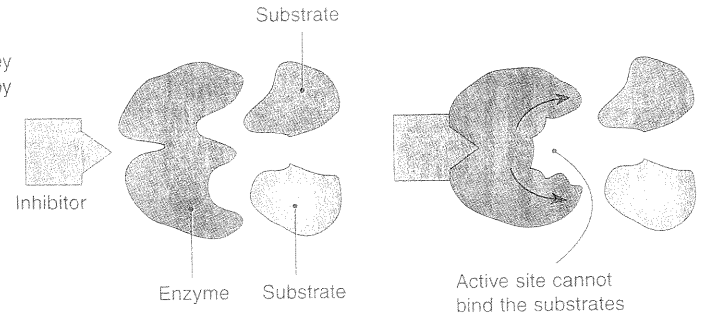
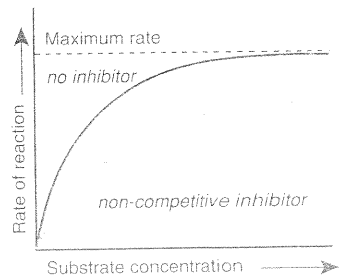


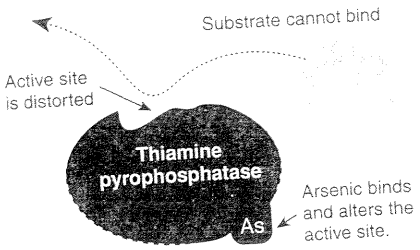
Figure 2 Effect of non-competitive inhibition on enzyme reaction rate at different substrate concentration



Poisons are Irreversible Inhibitors

Some enzyme inhibitors are poisons because the enzyme-inhibitor binding is irreversible. Irreversible inhibitors form strong covalent bonds with an enzyme. These inhibitors may act at, near, or remotely from the active site and modify the enzyme's structure to such an extent that it ceases to work. For example, the poison **cyanide** is an irreversible enzyme inhibitor that combines with the copper and iron in the active site of **cytochrome *c* oxidase** and blocks cellular respiration.

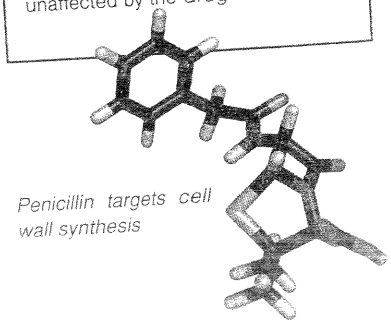
Since many enzymes contain sulfhydryl (-SH), alcohol, or acidic groups as part of their active sites, any chemical that can react with them may act as an irreversible inhibitor. Heavy metals, Ag^{+}, Hg^{2+}, or Pb^{2+}, have strong affinities for -SH groups and destroy catalytic activity. Most heavy metals are non-competitive inhibitors.



Arsenic and phosphorus share some structural similarities so arsenic will often substitute for phosphorus in biological systems. It therefore targets a wide variety of enzyme reactions. Arsenic can act as either a competitive or a non-competitive inhibitor (as above) depending on the enzyme.

Drugs

Many drugs work by irreversible inhibition of a pathogen's enzymes. Penicillin and related antibiotics inhibit transpeptidase, a bacterial enzyme which forms some of the linkages in the bacterial cell wall. Susceptible bacteria cannot complete cell wall synthesis and cannot divide. Human cells are unaffected by the drug.



Penicillin targets cell wall synthesis

1. Distinguish between **competitive** and **non-competitive** inhibition:

2. (a) Compare and contrast the effect of competitive and non-competitive inhibition on the relationship between the substrate concentration and the rate of an enzyme controlled reaction (figures 1 and 2 on the previous page):

   (b) Suggest how you could distinguish between competitive and non-competitive inhibition in an isolated system:

3. Describe how an **allosteric regulator** can regulate enzyme activity:

4. Explain why heavy metals, such as lead and arsenic, are poisonous:

5. (a) Using an example, explain how enzyme inhibition is exploited to control human diseases:

   ...organism, but not to humans: