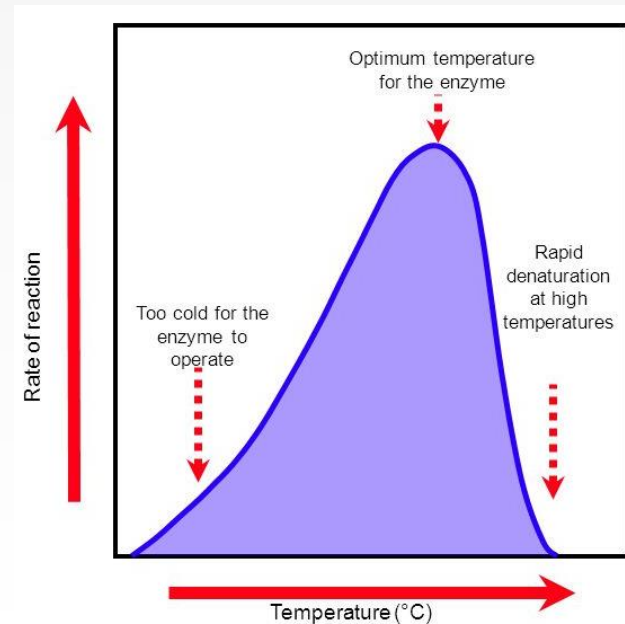
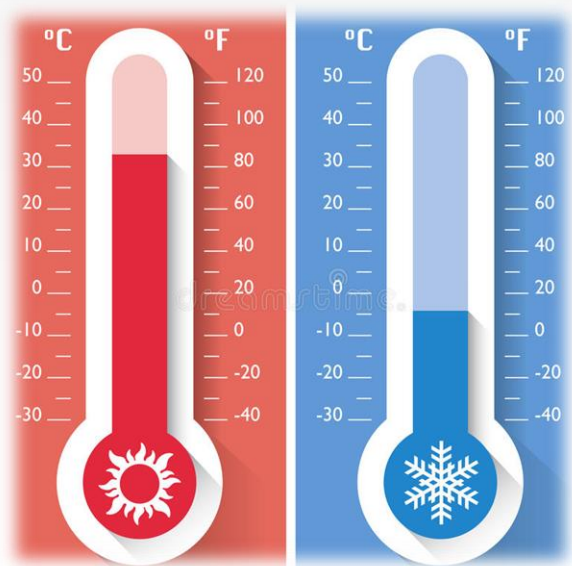
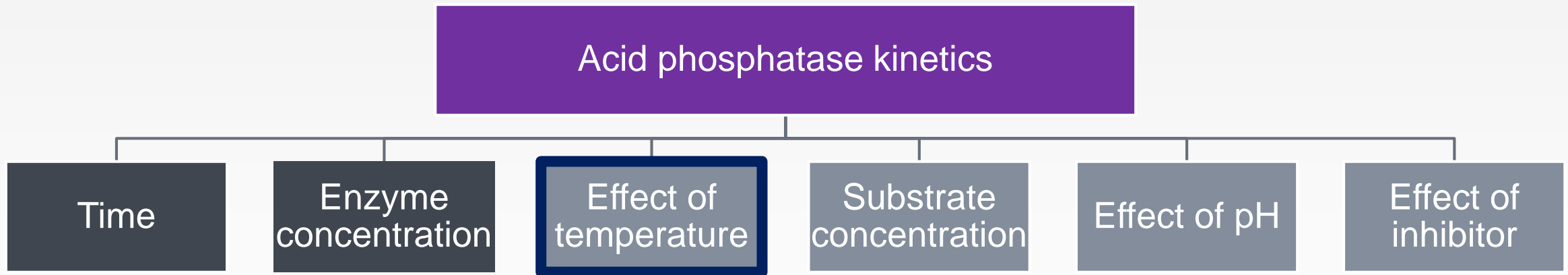


The effect of temperature on the rate of an enzyme catalyzed reaction



- In this experiment, we will continue to study acid phosphatase kinetics

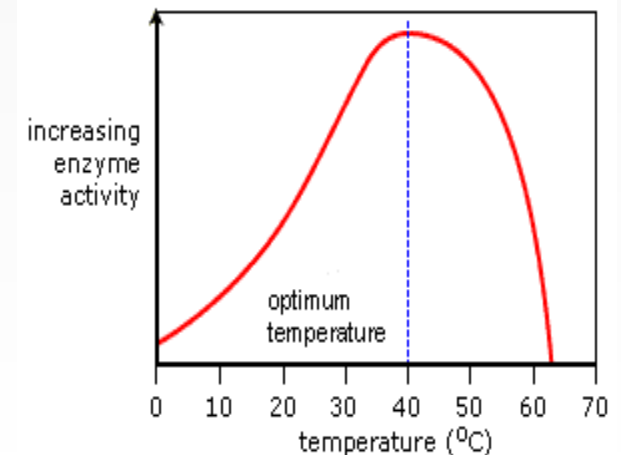
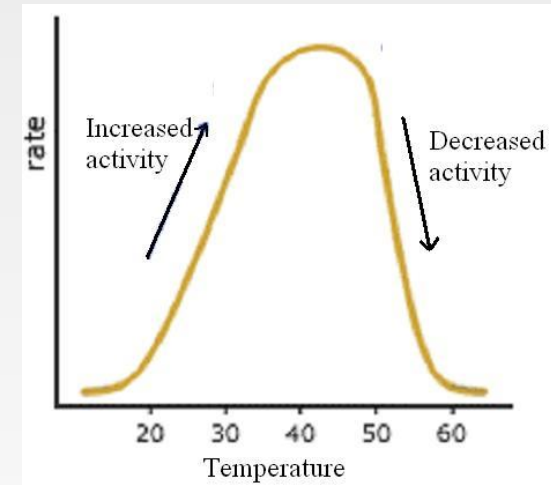


Objectives

- To establish the relationship between temperature and the rate of an enzyme catalyzed reaction.
- To determine the optimum temperature for such a reaction.

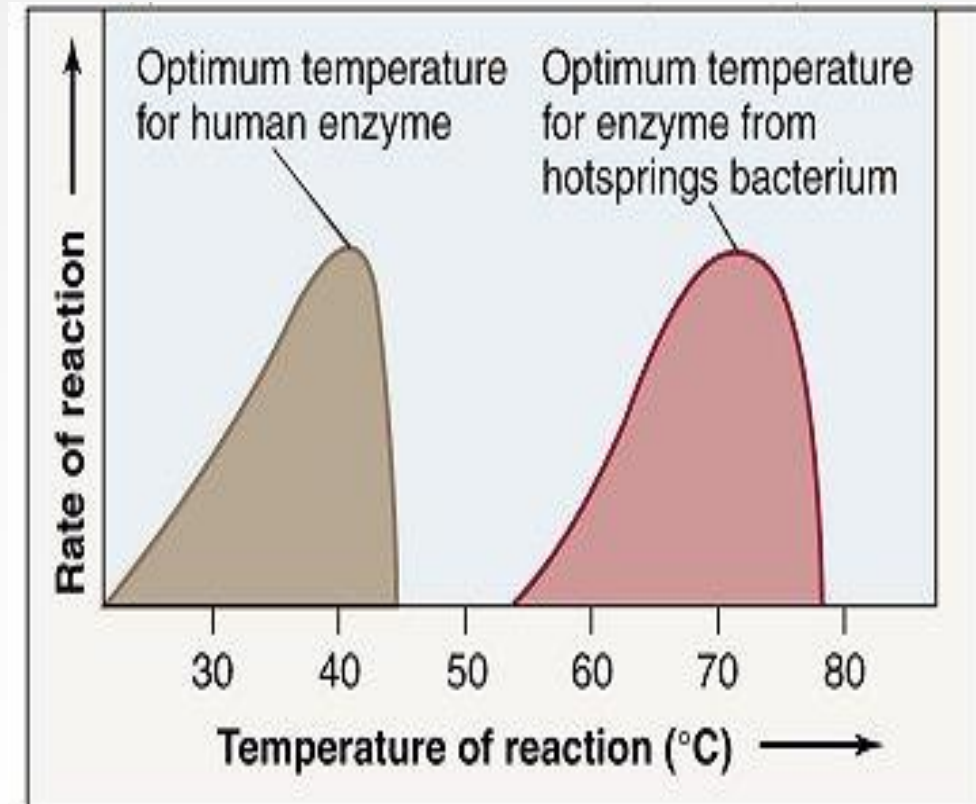
The effect of Temperature on Enzyme

- The rate of an enzyme catalyzed reaction is affected by changes in temperature
- Each enzyme has a temperature that it works optimally in called (**Optimum Temperature**)
- For most enzymes, the optimum temperature is at or above the temperature of the cells in which the enzyme is found in **vivo**.



Optimum Temperature

- Each enzyme has a temperature that it works optimally in, which **in humans is around 37** degrees Celsius; the normal body temperature for humans. However, some enzymes work really well at lower temperatures like 4 degrees Celsius
- and some work really well in higher temperatures like 95 degrees Celsius.

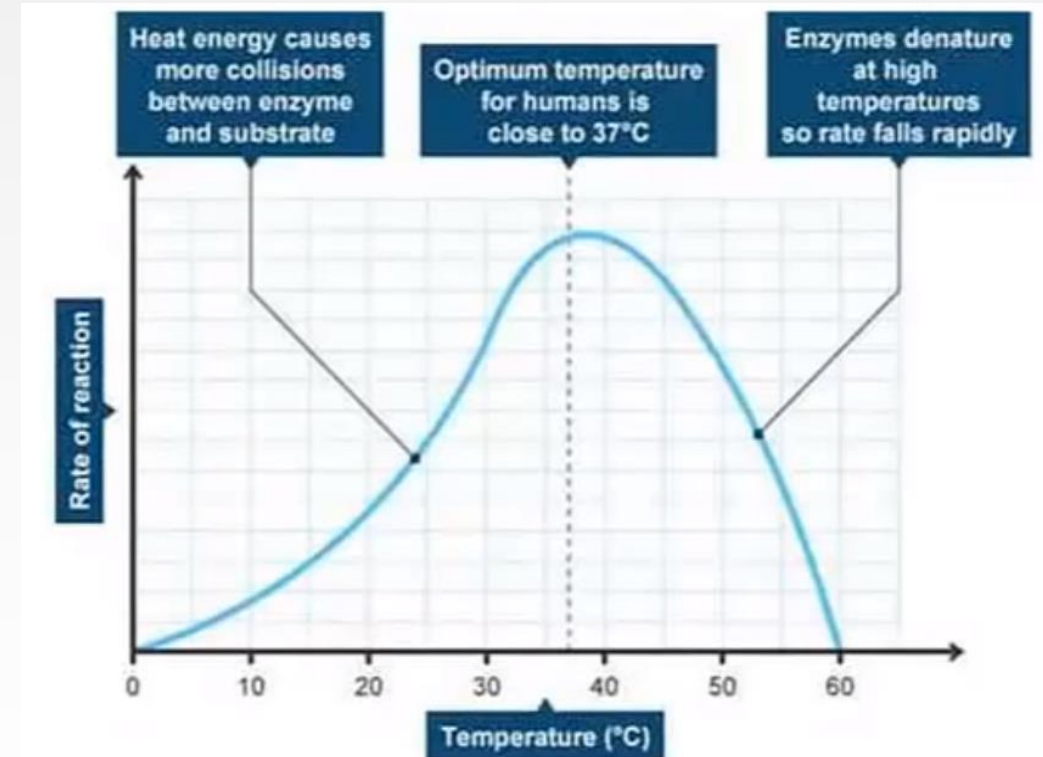


What is The effect of Temperature on the activity of Enzyme of most enzymes?

The rate of an enzyme catalyzed reaction increase with temperature up to maximum called optimum temperature

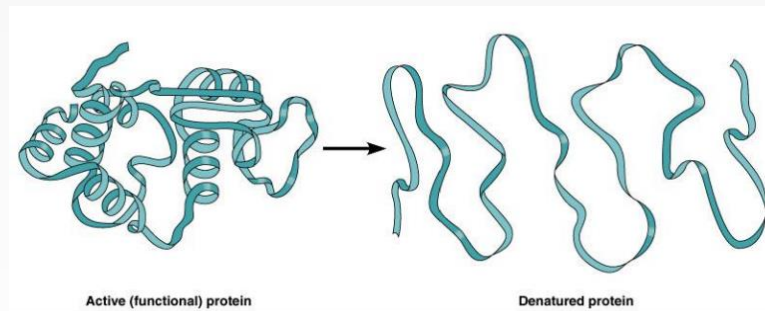
1-At suboptimal temperature, increasing temperature increase the kinetics energy of the reactant. As they move faster, they move likely to collide and interact with each other and the enzyme.

2-The rate continues to rise until reaches a peak at the optimum temperature

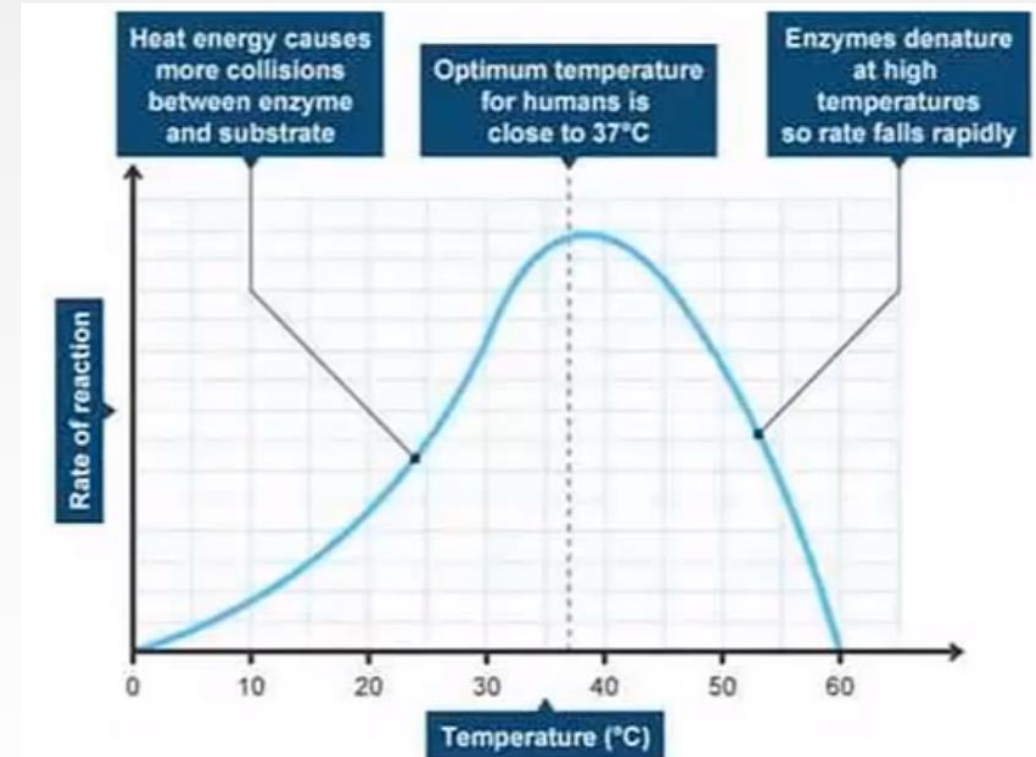


What is The effect of Temperature on the activity of Enzyme of most enzymes?

3-Above this temperature, the rate usually falls dramatically. This is because the increase energy cause bonds that maintain the enzymes shape to break, and the enzyme becomes **denatured**.(three dimensional structure will change)



- The changed shape means that the substrate can no longer fit into the active site, and enzyme activity is lost.



Method:

- This exercise illustrates the effect of increasing enzyme concentrations on reaction rate.
- You will perform a series of **5-minutes assays**, in which different temperature will be used

In order to detect the effect of Temperature you must fix all the component except the temperature	
Time (5 minutes)	constant
Enzyme concentration	constant
Temperature (degree)	Variable
pH (5.7)	constant

Method:

- The effect of the following temperature will be studied on Acid phosphatase:

Desired temperature (°C)	Method of preparation
4°C	Ice plus tap water in an ice bucket
25°C	Room temperature
37°C	Thermostatted water bath
65°C	Thermostatted water bath
80°C	Thermostatted water bath
100°C	Thermostatted water bath

Method:

- Label 12 assay tubes as the following:

Each should have its own Blank	
Blank-4C°	Test-4 C°
Blank-25 C°	Test-25 C°
Blank -37 C°	Test-37 C°
Blank-65 C°	Test-65 C°
Blank-80 C°	Test-80 C°
Blank-100 C°	Test-100 C°

Method:

- You must prepare the following for each tube(Blank + Test):

Chemical	Volume
1.0M sodium acetate buffer (pH 5.7)	0.5 ml
0.1M MgCl₂	0.5 ml
p-nitrophenyl phosphate	0.5 ml
Water	5 ml

- Place the tubes in the labeled temperature and let the temperature equilibrate for 5 min

Method-Con't

- For the blank you must first add 0.5 ml KOH then add the enzyme (we do not want any product to appear in the blank)
- For **TEST** , Add **0.5 ml of enzyme** extract to TEST and allow the reaction to proceed for **5 min**, using all the water bath temperatures described in the previous table
- Stop the reaction by the addition of **0.5ml of KOH** .
- *When all of the reaction mixtures have returned to room temperature determine the absorbance at 405 nm of each experimental tube against its own blank.*

Results :

Temperature	Absorbance 405 nm	Velocity (μ mole of PNP/min)
0		
25		
37		
65		
80		
100		

Plot a graph illustrating the effect of different temperatures on the rate of the reaction.

Calculations:

$$\text{Velocity (V)} = (A \times 10^6) / (E \times \text{time}) = \text{ }\mu\text{mole of PNP/min}$$

A= absorbance

E= extension coefficient= 18.8×10^3

Time = 5 min

Discussion

- Discuss the shape of the graph. And From the curve, explain and discuss the relationship between the activity of acid phosphatase and temperature.
- Define the optimum temperature and determine it from the curve.