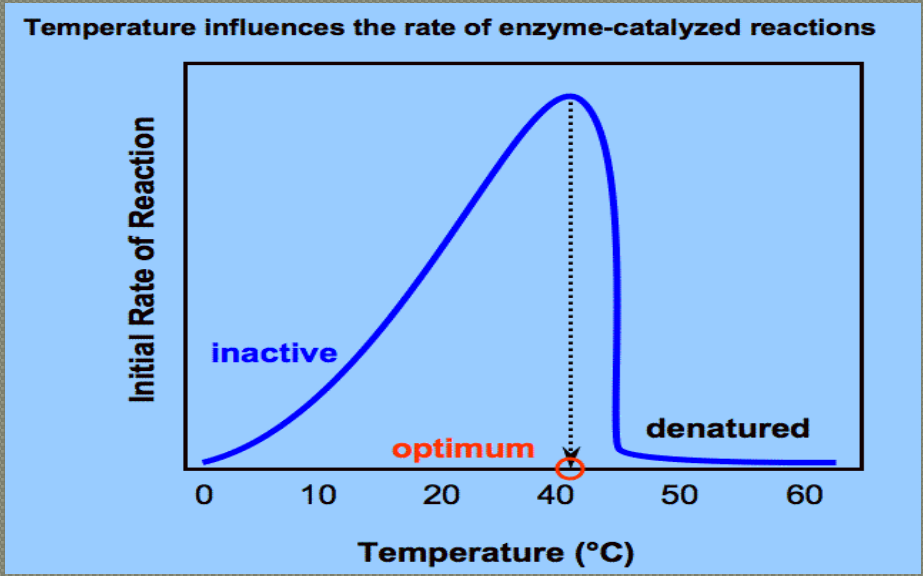
The Effect of Temperature on the Rate of An Enzyme Catalyzed Reaction

Enzymology

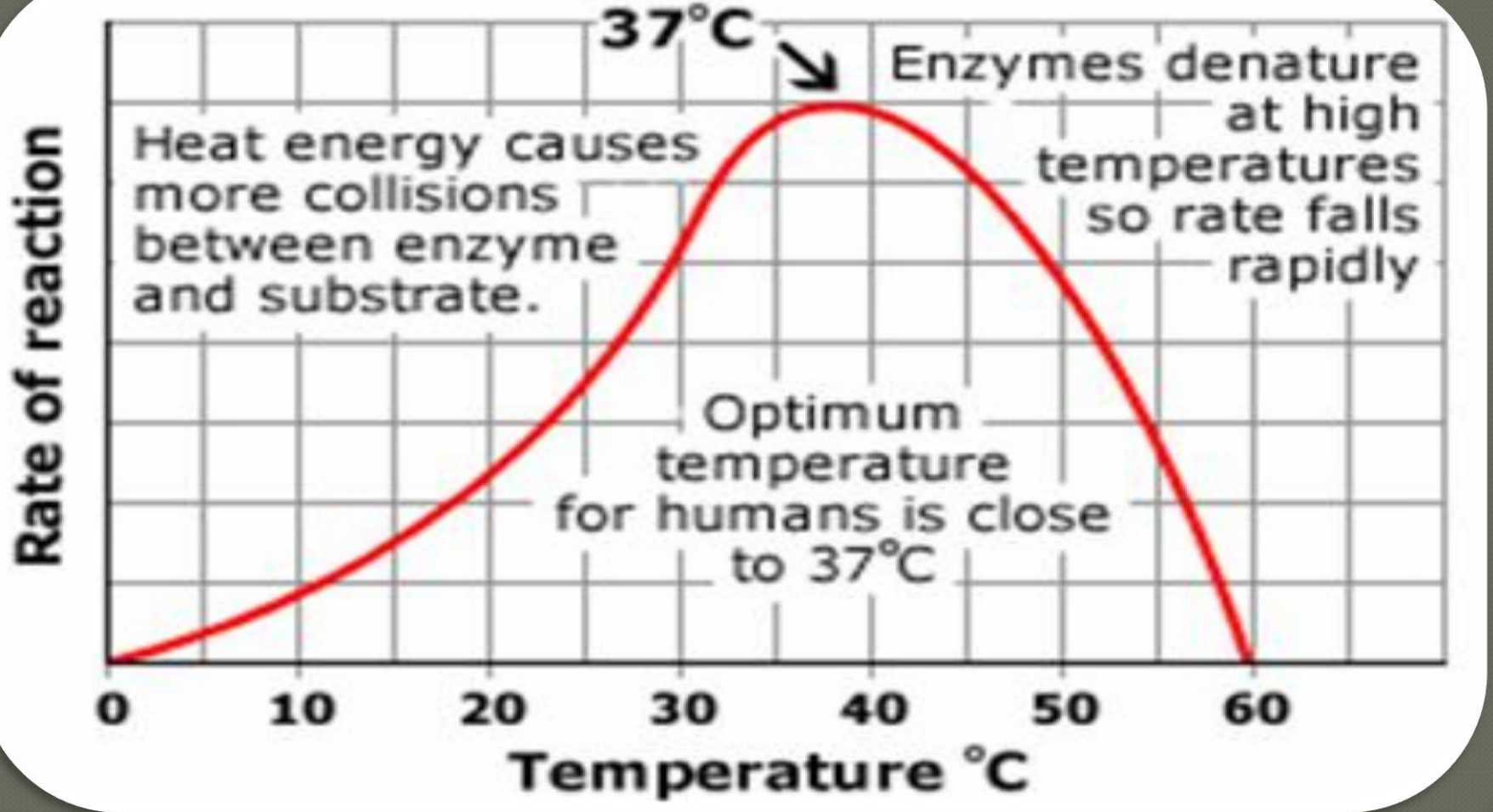


Principle

The rate of an enzyme catalyzed reaction is affected by changes in temperature.

- At low temperature (0˚C), the rate of reaction is low. As the temperature is increased, the rate of reaction increase until an optimum temperature is reached. Within this temperature range, the rate of reaction is approximately doubled for every 10 ˚C rise in temperature.
- **With further rise in temperature, above the optimum temperature,** ***the rate of reaction decreases due to denaturation of the enzyme protein and hence loss of activity*****.**

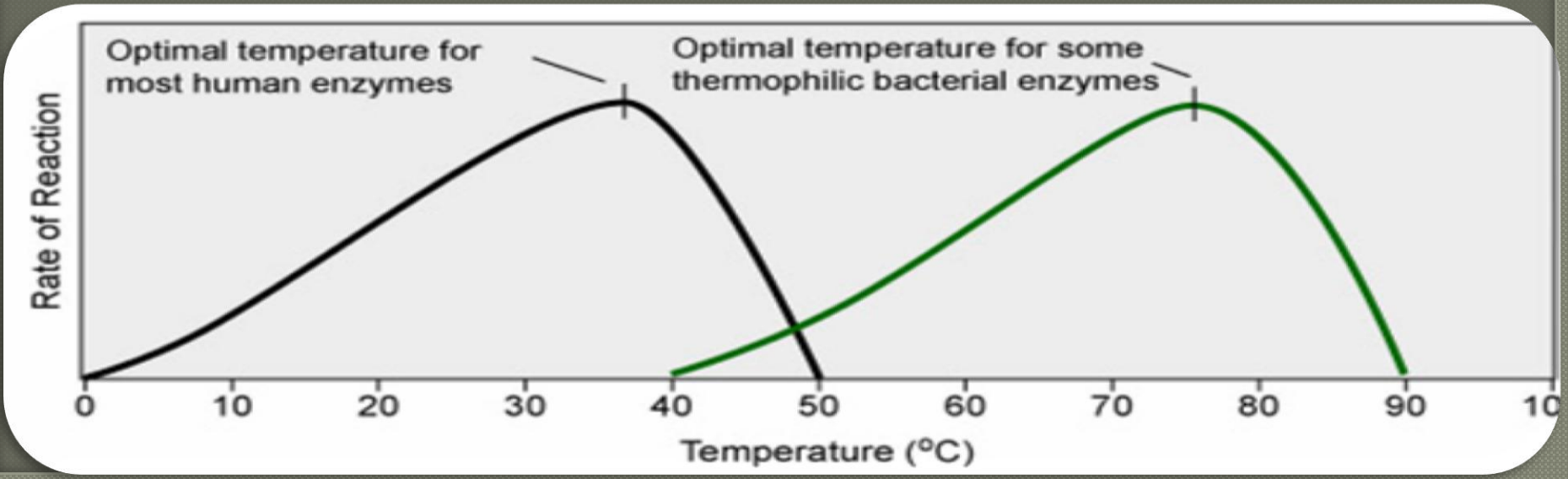
The relationship between the rate of the enzymatic reaction and the temperature is shown in the figure below



Optimum Temperature

is the result of the balance between the rate of an increase in the enzyme activity on the one hand and the rate of decrease due to denaturation on the other.

Most enzymes are inactivated at temperatures above 60 ˚C . For most enzymes, the optimum temperature is at or above the temperature of the cells in which the enzyme is found in vivo.



Materials

- Test tubes
- Pipettes
- Cuvettes
- Ice , Adjusted Water bath at different temperature
- Stopwatch
- Spectrophotometer
- Acid phosphatase enzyme
- 0.05 M PNPP
- 1 M sodium acetate buffer(PH 5.7)
- 0.1 M MgCl2
- 0.5 M KOH

Method

Desired temperature (˚C)	Method of preparation
0-4˚C	Ice plus tap water in an ice bucket
30˚C	Thermostatted water bath
37˚C	Thermostatted water bath
50˚C	Thermostatted water bath
80˚C	Hot tap water
100˚C	Boiling water bath

- Label 14 assay tubes (8(blanks), 8 tests) and into each of them pipette 0.5ml of :

buffer (pH 5.7),
0.5ml of MgCl2,
0.5ml of Enzyme

Blank tubes has no substrate.

- Place the tubes in a water bath maintained and let the temperature equilibrate for 5 min.
- Add 0.5 ml of p-NPP to tubes and allow the reaction to proceed for 5 min before stopping it with the addition of 0.5ml of KOH . Tube which serves as a reagent blank, should be treated in the same fashion except that 0.5ml of distilled water should be added to the reaction mixture instead of substrate.
- 6.Repeat steps using all the water bath temperatures described in the previous table. 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 tube A.

Result

temperature (˚C)	Absorbance at 405 nm	Velocity (µ moles of P-NP/minute)
0-4˚C		
30˚C		
37˚C		
50˚C		
80˚C		
100˚C		

Calculation

- *Convert absorbance data to velocity data.*
- *Plot a graph illustrating the effect of different temperatures on* the rate of the reaction.