Model Questions And Answers

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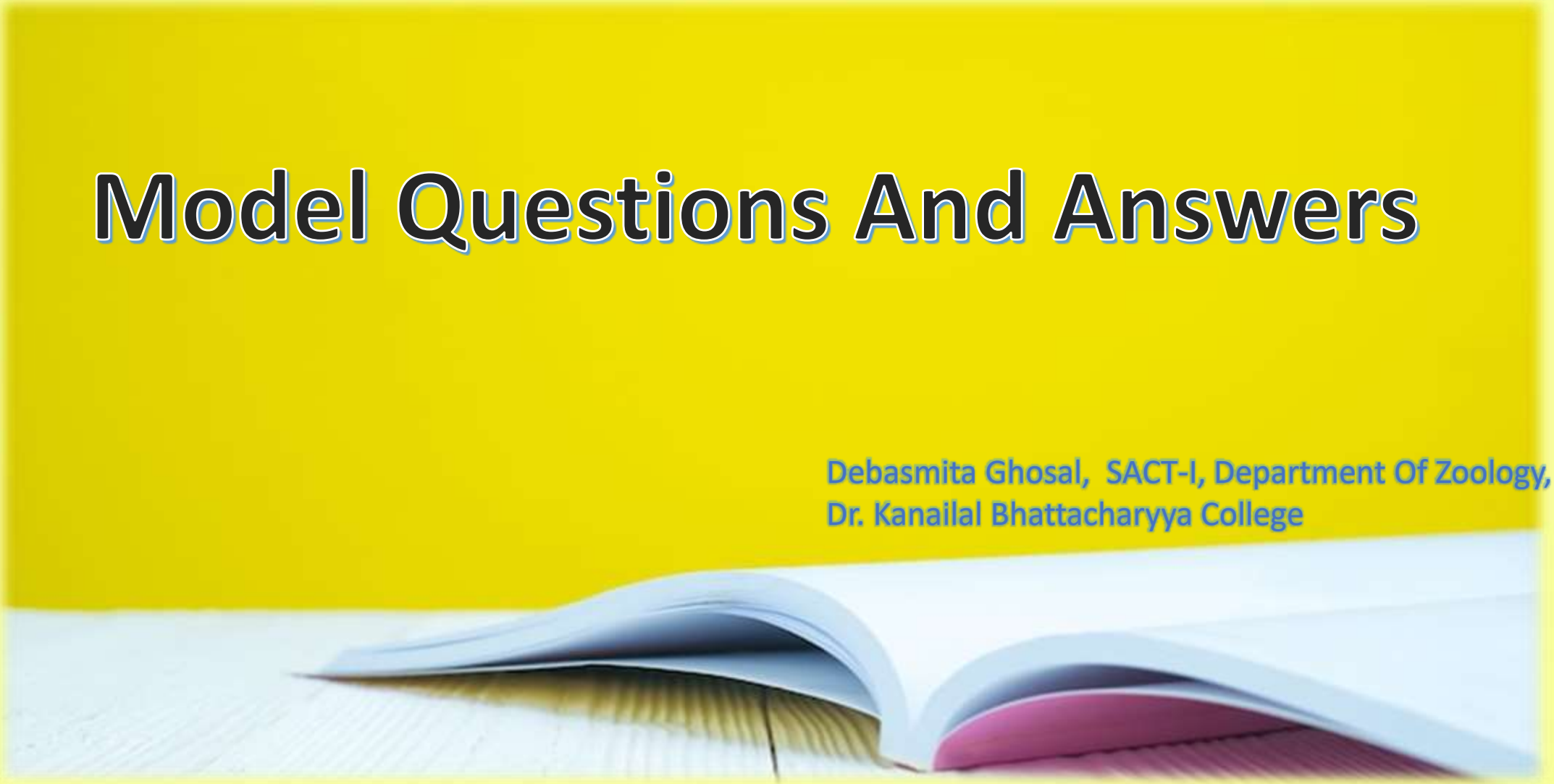
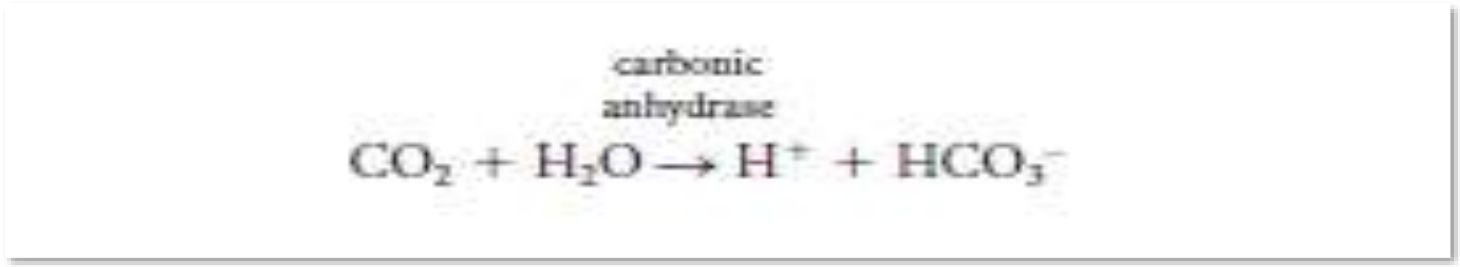


Q) What do you mean by chloride shift and reverse chloride shift?

- The phenomenon of exchange of chloride ions between the blood plasma and the red blood cells when carbon dioxide enters the plasma from the tissues is known as chloride shift and their return to the plasma from RBC when the carbon dioxide is discharged in the lungs is known as reverse chloride shift. These phenomena play a major role both in maintenance of blood pH and in transport of carbon dioxide.
- As a result of catalysis by carbonic anhydrase within the red blood cells, large amounts of carbonic acid are produced as blood passes through the systemic capillaries.
- The build up of carbonic acid concentration within the red blood cells favors the dissociation of these molecules into hydrogen ions (protons, which contribute to the acidity of a solution) and HCO_3^- (bicarbonate), as shown by the following equation:



- The hydrogen ions (H^+) released by the dissociation of carbonic acid are largely buffered by their combination with deoxyhemoglobin within the red blood cells. Although the unbuffered hydrogen ions are free to diffuse out of the

red blood cells but more bicarbonate diffuses outward through the HCO_3^-–Cl^- Antiporter (Band3 protein) of RBC into the plasma than does H^+ due to the relative impermeability of RBC membrane to H^+ .

- The "trapping" of hydrogen ions within the red blood cells leads to the efflux of HCO_3^-unaccompanied by a comparable outward diffusion of positively charged ions creating an electrical gradient i.e. the inside of red blood cell gains a net positive charge.
- Chloride ions (Cl^-), the dominant plasma anions then diffuse into the red blood cells down this electrical gradient to restore electric neutrality.
- This exchange of anions as blood travels through the tissue capillaries is called the **chloride shift.**
- When blood reaches the pulmonary capillaries , deoxyhemoglobin is converted to oxyhemoglobin. Because oxyhemoglobin has a weaker affinity for H^+ than does deoxyhemoglobin,hydrogen ions are released within the red blood

cells. H^+ reacts with HCO_3^- to form Carbonic acid, which is catalyzed by carbonic anhydrase to carbon dioxide and water.

$$H_2CO_3 \xrightarrow[\text{low } P_{CO_2}]{\text{carbonic anhydrase}} CO_2 + H_2O$$

- The produced CO_2 goes out to the alveolar air. So [$HCO3^-$] in R.B.C decreases hence $HCO3^-$ from the plasma enters to have the same fate and it continues. Chloride ions which entered in the chloride shift now come out of RBC to maintain electroneutrality and this phenomenon is called the **reverse chloride shift**.

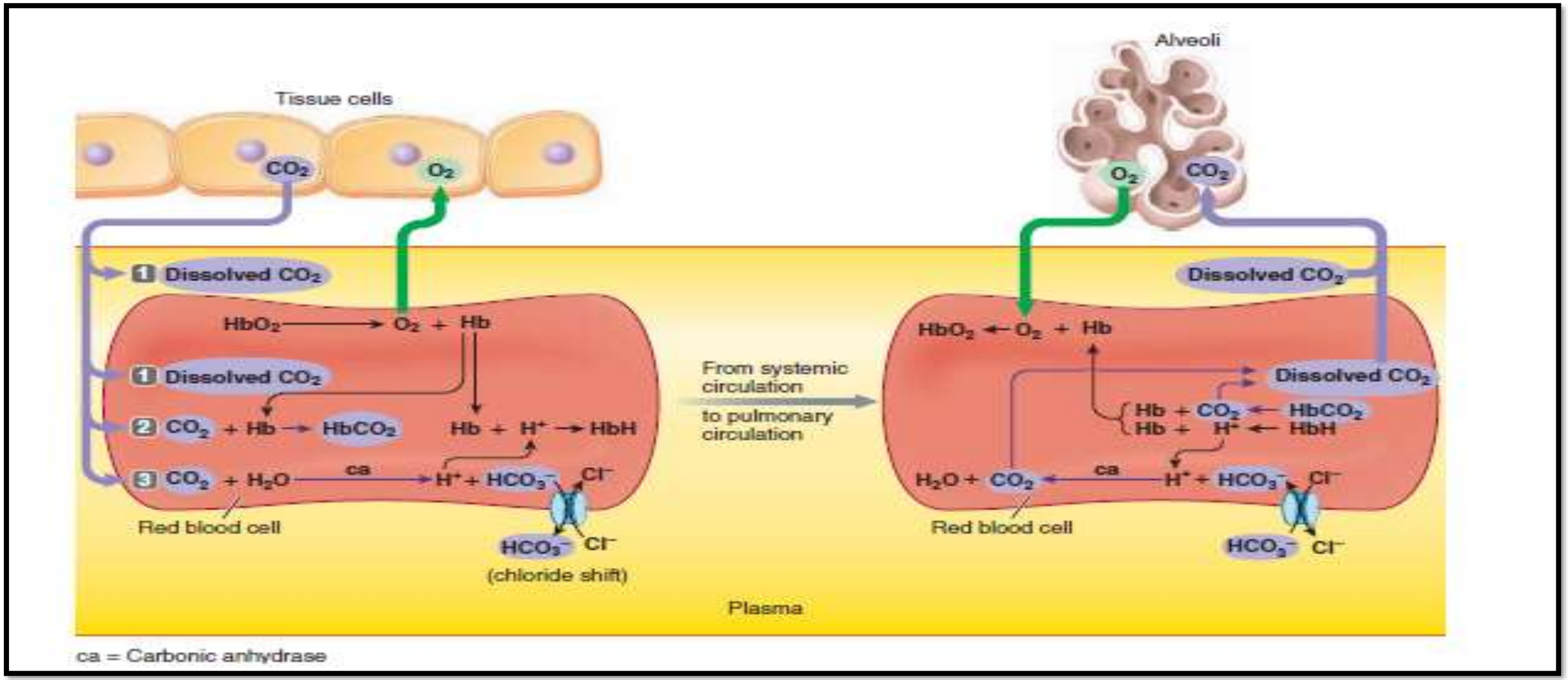
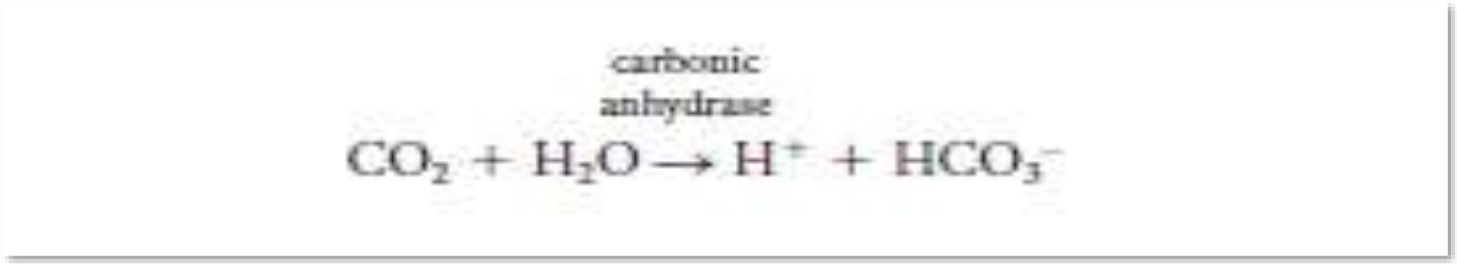


Fig 1 : Chloride shift and reverse chloride shift occurring during carbon dioxide transport of blood.

Q) What is Bohr effect?

Ans: The influence of CO_2 and acid on the release of O_2 from hemoglobin is known as the Bohr effect. Increased CO_2 in blood produces increased amount of H^+ decreasing the pH inside RBC.



This increased [H^+] alters the configuration of Hb molecules and accessibility to O_2 is reduced. So affinity of Hb to O_2 is decreased and O_2 is released. Thus the oxygen dissociation curve of haemoglobin is shifted to the right by a lowering of pH or increased [H^+] or increase in partial pressure of carbon dioxide indicating a greater unloading of oxygen.

Significance: Since the pH can be decreased by CO_2 the Bohr effect helps to provide more oxygen to the tissues when their carbon dioxide output is increased by a faster metabolism. That is the Bohr effect is useful during physical exercise when more CO_2 is produced ensuring more O_2 delivery to active tissues.

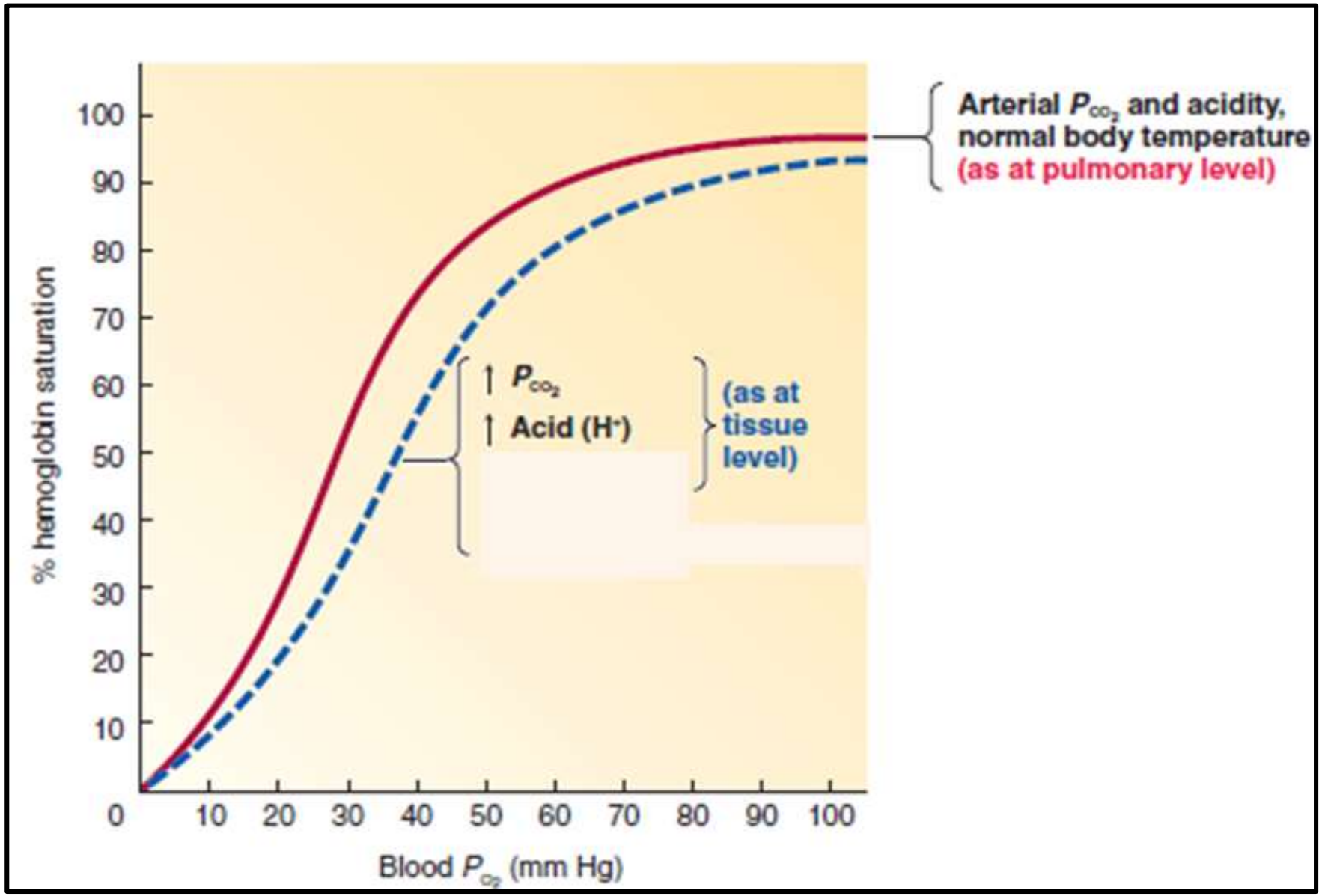
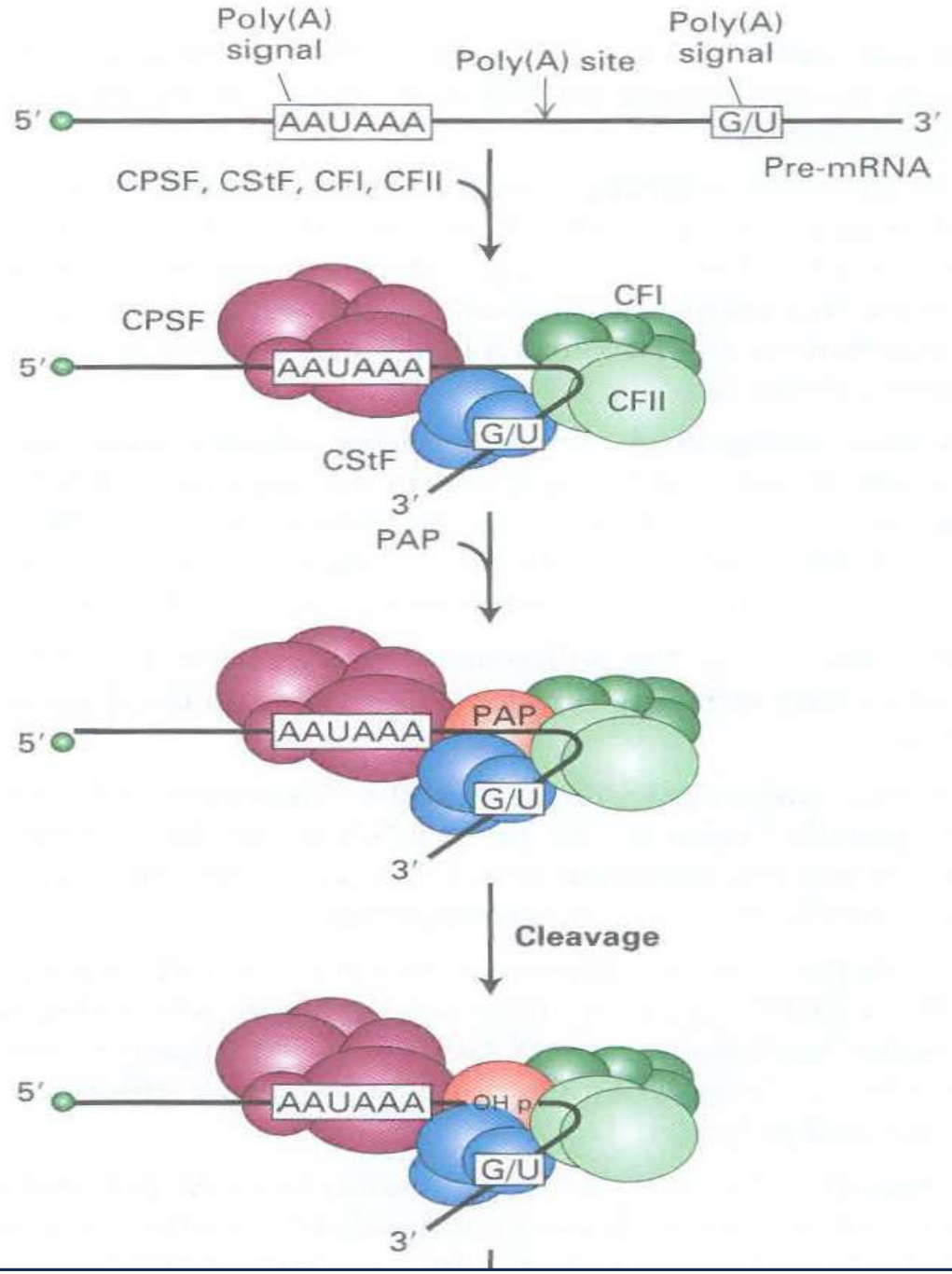


Fig 2: Bohr effect

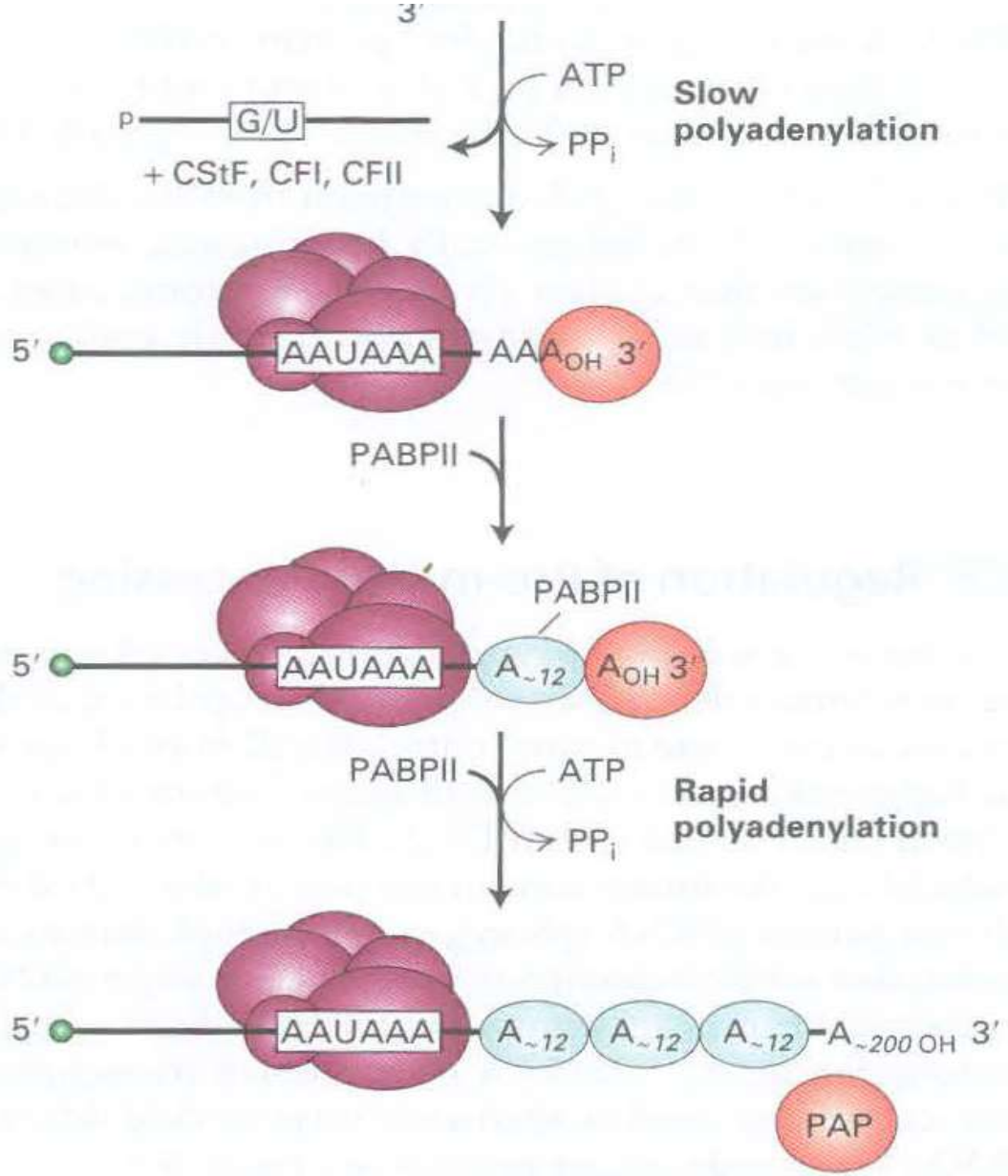
Q. Describe the process of cleavage and polyadenylation in eukaryotes.
Ans:

- In Eukaryotes once the RNA polymerase II has reached the end of a gene, it encounters specific signal sequences upstream AAUAAA poly(A) signal and a downstream GU- or U-rich sequence that, after being transcribed into RNA, trigger the transfer of certain enzymes and protein factors to that RNA that generate the 3’ end of pre-mRNA by a process known as cleavage followed by a post transcriptional modification in which 50 to 250 or more adenine nucleotides are added at the 3’ end of pre-mRNA generated with the help of an enzyme called poly-A polymerase (PAP) forming a **poly(A) tail** and is termed as polyadenylation.
- Once poly-A signals transcribed into RNA, two protein complexes carried by the CTD of polymerase as it approaches the end of the gene: CPSF (cleavage and polyadenylation specificity factor)and CSTF(cleavage stimulation factor) are transferred to the RNA .
- Once CPSF and CSTF are bound to the RNA, other proteins are recruited as well, leading initially to RNA cleavage and then polyadenylation.
- In the process of polyadenylation the PAP enzyme uses ATP as a precursor and adds the nucleotides using the same chemistry as RNA polymerase but unlike RNA Polymerase it does so without a template i.e. the Poly(A)tail is not encoded by the DNA.
- The sequencial process of cleavage and polyadenylation involves the following steps:



The Cleavage and polyadenylation specificity factor (CPSF) composed of five different polypeptides, binds to the upstream AAUAAA poly(A) signal and heterotrimer CStF interacts with a downstream GU- or U-rich sequence and with bound CPSF, forming a loop in the RNA. The complex is stabilized by the binding of heterotetramer called cleavage factor I (CFI) and heterodimeric cleavage factor II (CFII). An -150-kDa protein called symplekin is thought to form a scaffold on which these cleavage/polyadenylation factors assemble.

Binding of poly(A) polymerase (PAP) then stimulates cleavage at a poly(A) site, which usually is 10-35 nucleotides 3' of the upstream poly(A) signal. The 73-kD subunit of CPSF acts as as the cleavage endonuclease.



The cleavage factors are released, as is the downstream RNA cleavage product, which is rapidly degraded.

Bound PAP then adds - 12 A residues at a slow rate to the 3'-hydroxyl group generated by the cleavage reaction.

Binding of poly(A)-binding protein II (PABPII) to the initial short poly(A) tail accelerates the rate of addition by PAP.

After 200-250 A residues have been added, PABPII signals PAP to stop polymerization.

Q. Write a short note on Somatic Cell Nuclear Transfer Method of animal cloning.

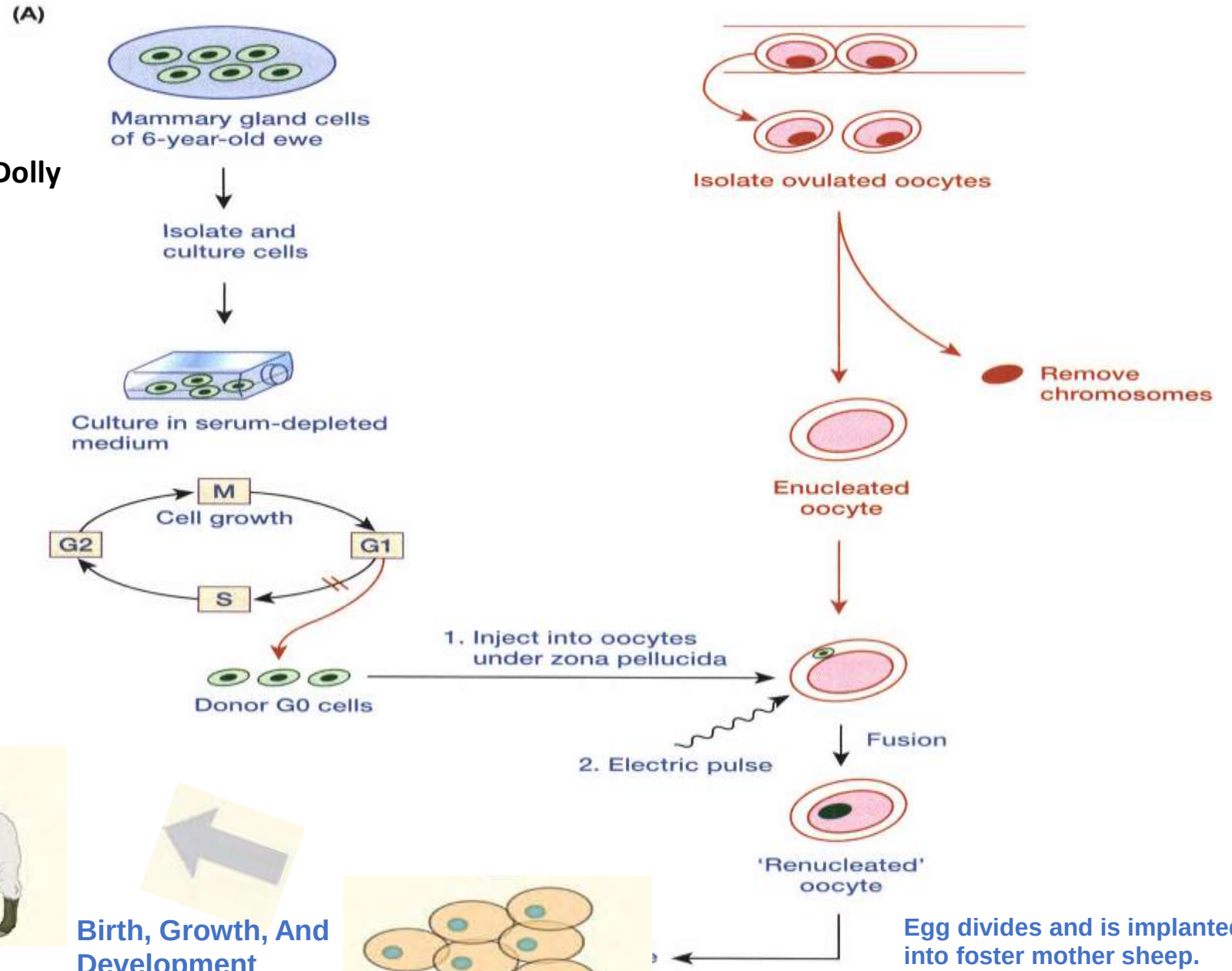
Ans:

• Somatic Cell Nuclear transfer (SCNT) is the transplantation of a diploid nucleus from the donor somatic cell to the enucleated oocyte, the later reprograms the somatic nucleus to recapitulate the whole of development despite the differentiated state of the donor cell.
• This recipient diploid oocyte mimics as zygote and begins to divide, specialize, and form a hollow sphere of cells, called a blastocyst. The blastocyst has an outer layer of cells called the trophectoderm, a hallow space inside called the blastocoel, and cluster of cells called the inner cell mass (ICM).
• The transplantation of the blastocyst into the uterus of an animal can lead to the successful birth of a cloned animal, and is called reproductive cloning.
• SCNT enabled both the generation of transgenic animals and, more importantly, gene-targeting.

- In early 1996, at the Roslin Institute in Scotland, the world's first cloned animal, Dolly the was born by this method and was soon extended to other species.
- In this method extensive selection and screening of donor cells ensure that 100% of offspring carry solely and precisely the intended modification.
- Site-specific endonucleases, such as zinc finger nucleases, transcription activator-like effector nucleases, and the CRISPR/Cas9 system facilitate the introduction of double strand breaks (DSBs) into predetermined sites within the host genome to trigger DNA repair mechanisms, thus enabling efficient genome engineering.

- Genome editing in combination with SCNT allows for comprehensive *in vitro* off-target analysis prior to the generation of animals, avoiding undesirable mutations as well as mosaicism.The individual strengths and weaknesses of both approaches complement each other well, and together, they provide an efficient toolkit for the generation.
- The Scheme for Cloning Dolly is discussed below.In Dolly's case, cultured udder cells from the donor animal were starved so that both the cell and the DNA stopped dividing (i.e., the cells entered the G_0 stage of the cell cycle). Probably some modifications take place in starved condition, including demethylation, that convert the DNA back to a form resembling that of an embryonic cell. When the resting G_0 nucleus was placed in an egg cell whose own nucleus had been removed, it started dividing again. The egg was then transplanted into a female animal, where it developed into an embryo and finally into a new born transgenic sheep.

Fig : Scheme for Cloning Dolly



Q. What is a coenzyme?

Ans: A coenzyme is a small,heat stable organic cofactor which binds very loosely and transiently to the apoenzyme-usually by noncovalent bond,participates in a single reaction catalyzed by that enzyme and is released in an altered form at the end of it,binds next to another apoenzyme to participate in the reaction catalysed by it and get reconverted to its original form.
Ex-NAD^+ of Glyceraldehyde-3-Phosphate Dehydrogenase(E1).
Coenzymes NAD^+ receives a Hydride ion(H^-) from a substrate to be reduced to NADH by the action of one Dehydrogenase,and is subsequently oxidised back by a second Dehydrogenase into NAD+ by donating the H- ion to a second substrate.

Q. What is a prosthetic group?

Ans: Prosthetic group is usually considered to be an organic cofactor covalently bound to the apoenzymes,do not dissociate from them easily,and go on participating repeatedly in the successive reactions catalyzed by the same respective enzyme without fleeting to other apoenzymes.
Ex-PLP,the Prosthetic group of Transaminases,first receives an amino group from an acid to change into Pyridoxamine phosphate(PMP) which next donates the amino group to an α-Ketoacid to change back to PLP under the action of the same enzyme.

Q. State the assumptions of Michaelis-Menten Equation.

Ans: Assumptions of Michaelis-Menten equation modified by Haldane includes the following:

1. A single substrate enzyme catalyzed reaction is considered where there is just one substrate binding site/enzyme and only one ligand-the substrate. Coenzyme inhibitors and activators are ignored.
2. A complex is formed between the enzyme and the substrate and this breaks down to form product(s).
3. Investigations are restricted to the initial period of the reaction.
4. Equilibrium between E,S and ES complex was almost instantly set up and maintained; the breakdown of ES complex to product being too slow to disturb this equilibrium.
5. The amount of substrate bound is small so that the concentration of the free substrate is the same as the total present.
6. The total amount of enzyme present is the sum of the free enzyme and that bound in ES complex.
7. A steady state is rapidly attained and exists for the duration of the measurement. (Briggs & Haldane)
8. Initial velocity (V_0) is determined by the rate of breakdown of ES.

Q. What happens when [S] << K_m in a single substrate enzymatic reaction?

Ans : From Michaelis-Menten equation we know

$\therefore V_0 = \frac{V_{max}[S]}{K_m + [S]}$ where V_0 = Initial Velocity of the enzymatic reaction

V_{max} = Maximum velocity of the enzymatic reaction

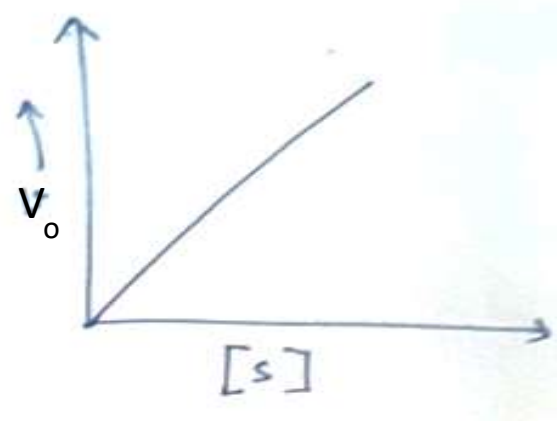
[S] = Substrate concentration

K_m = Michaelis Menten Constant.

At substrate concentration far below K_m,

i.e. at [S] << Km , Km + [S] ≈ K_m

∴ At substrate concentration far below Km, the Michaelis-menten equation becomes



First order reaction kinetics graph

$\therefore V_0 = \frac{V_{max}[S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m} = K[S]$ where K= constant= $\frac{V_{max}}{K_m}$ [Since for a particular enzyme acting on a specific substrate both V_{max} and K_m are constants]

$\therefore V_0$ = constant[S]

$\therefore V_0 \propto [S]$

This shows that with very low substrate concentration the single substrate enzymatic reaction becomes a first order reaction with its rate depending on the concentration of the single reactant. Therefore at substrate concentrations far lower than the K_m , V_o is directly proportional to [S]; as a result of which the initial segment of the hyperbolic kinetics plot of Michaelis-Menten equation is linear.