CASE

1

Classical Galactosemia

A 4-month-old male child presents with the history of repeated loss of consciousness and refusal to feed specially milk and milk-containing diet. On examination, baby was found to be mild icteric and bilateral cataract* was detected. Liver was palpable below costal margin.

Following are the results of various laboratory investigations:

- Blood sugar: 72 mg/dl (normal random blood glucose = 80–140 mg/dl)
- Plasma-free galactose: 129 mg/dl (normal = <20 mg/dl)
- Serum uric acid: 8.4 mg/dl (normal = 2.5–7.2 mg/dl)
- Blood lactic acid: 4.8 mmol/L (normal = 0.5 to 1.0 mmol/L)
- RBC galactose-1-phosphate level: 54 mg/dl (normal = <1 mg/dl)
- RBC galactose-1-phosphate uridyltransferase activity was absent.

QUESTIONS

Q.1. What is the probable diagnosis in this case? What is the biochemical defect in this disease?

Q.2. Explain the biochemical reason for the above clinical signs and symptoms.

Q.3. What is the urinary finding in this case?

Q.4. What is the treatment regime suggested for this child?

Explanations

Ans.1. This child is suffering with **classical galactosemia** which is due to deficiency of enzyme: **'Galactose-1-phosphate uridyltransferase' (GaliPUT).** This enzyme is responsible for conversion of galactose-1-phosphate to glucose-1-phosphate. It is an autosomal recessive disorder. The pathway of galactose metabolism is illustrated in Fig. 1.

Ans.2. Biochemical explanation of clinical signs and symptoms is as follows:

a. **Hepatomegaly*:** Lack of this enzyme impairs galactose metabolism and results in accumulation of **galactose-1-phosphate** in liver which results in **hepatomegaly*.**

b. **Recurrent hypoglycemic attack:** Accumulated galactose-1-phosphate inhibits glycogen phosphorylase enzyme which is manifested as **recurrent hypoglycemic**

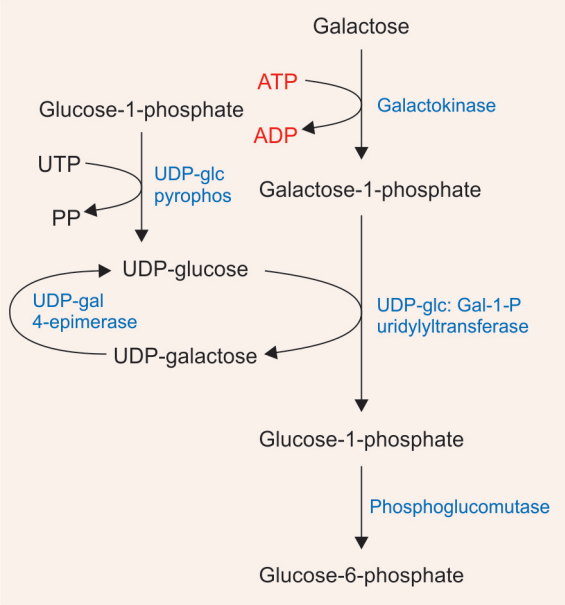

Fig. 1: Biochemical pathway involved in galactose metabolism

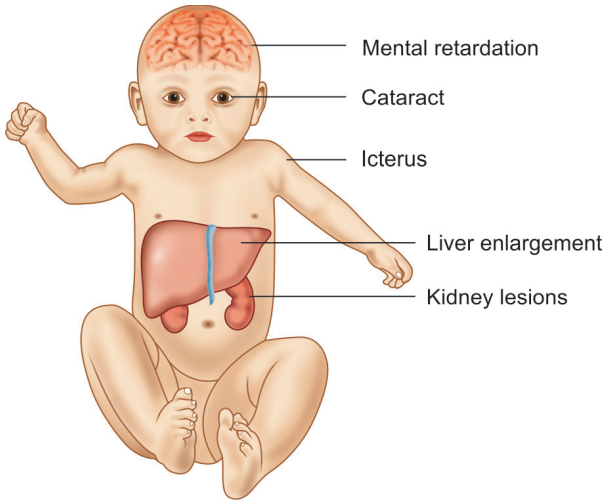

Fig. 2: Key manifestation of classical galactosemia

episodes. This is due to the fact that glycogen phosphorylase is the key enzyme in the liver to supply glucose in blood by glycogenolysis at the time of need. Once inhibited, this enzyme does not run the glycogenolysis and results in recurrent hypoglycemia.

c. **Cataract*:** Cataract in the baby is due to reduction of unmetabolized galactose in the lens of the eye in **polyol pathway**, where **aldol reductase** converts galactose to

galactitol which initiates cataract formation. Cataract is bilateral and in most of the cases, cataract is mild and transient and is reversible provided dietary treatment with lactose- and galactose-free diet, starts early within 20 days of life.

Ans.3. Galactose is not metabolized in galactosuria. This results in higher level of galactose circulating in blood which gets filtered out in the urine.

Benedicts test done in urine of such patients gives positive result as galactose is a reducing carbohydrate.

Ans.4. **Treatment of this child is lactose and galactose-free diet. This regime should be started within 10 days of life. Delay in starting the treatment results in organ damage and low IQ.** Despite adequate treatment from an early age, children with classic galactosemia remain at increased risk for developmental delays, speech problems and motor dysfunction.

Childhood **apraxia* of speech** and **dysarthria*** require expert **speech therapy.** Cataract may require surgery. If left untreated, galactosemic babies are prone to develop *E. coli* infection and may land up in sepsis and death.

Other important points related to this case:

- *Food items need to be avoided in galactosemic baby*: Breast milk, cow milk, casein and whey-containing food, medications having galactose and lactose to be avoided in galactosemia.
- Prenatal genetic diagnosis is possible in cultured amniotic fluid for **galactose-1-phosphate uridyltransferase enzyme assay.**

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Word Meaning

Hepatomegaly: Enlargement of liver.

Cataract: A disease which causes dense and cloudy lens of eye which impairs vision.

Apraxia: Inability to perform certain task.

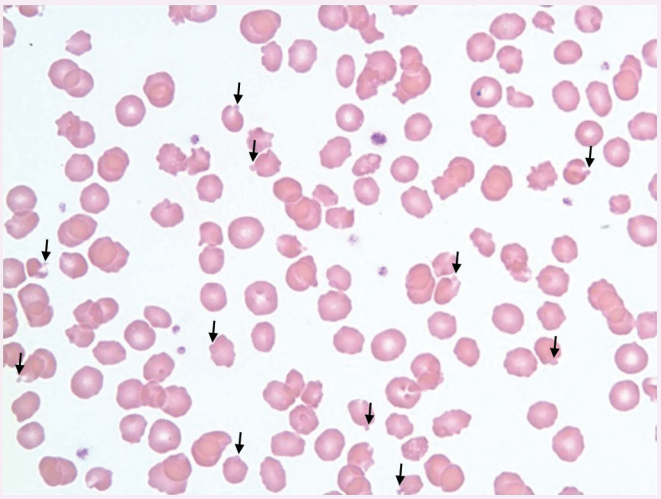
Dysarthria: Difficulty in articulation of speech.

CASE

2

Glucose-6-Phosphate Dehydrogenase Deficiency

A 23-year-old African boy is brought with fever, chills and rigors*; on examination, spleen was palpable*, **antimalarial drug primaquine** was given. After two days, patient visit again with history of fatigue, breathlessness, pale skin, passing dark-colored urine. He also reported having abdominal discomfort. Peripheral smears showed presence of Heinz bodies and few bite cells and blister cells (Fig. 1a and b).



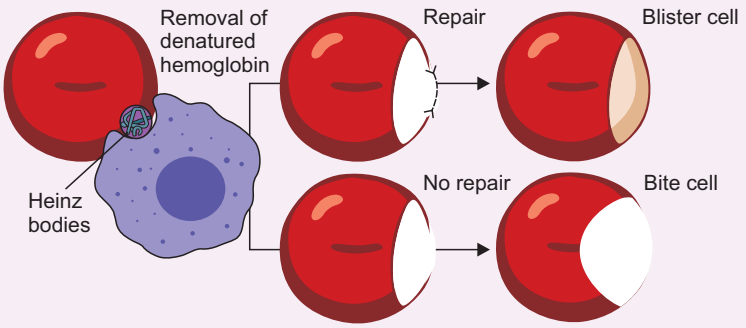


Fig. 1a: Heinz bodes

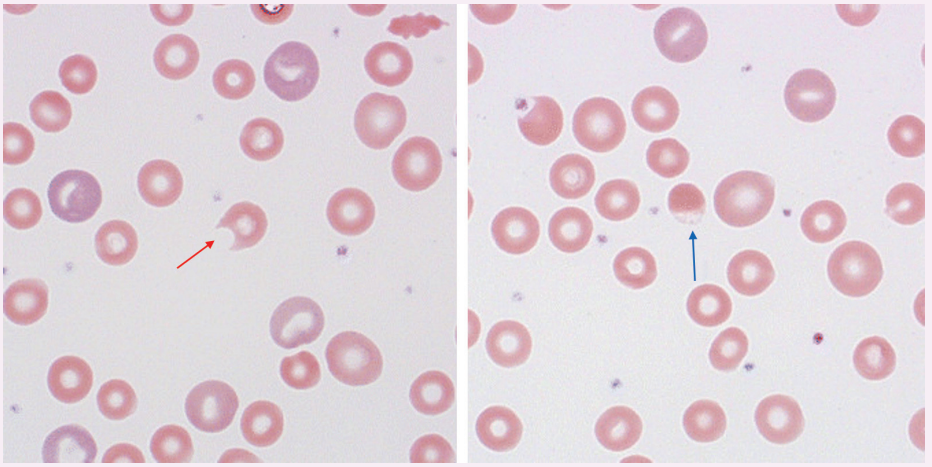
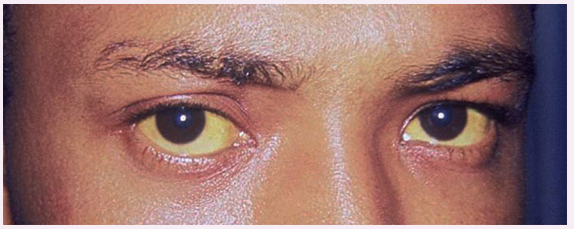


Fig. 1b: Bite cell and blister cell

Following are the results of various laboratory investigations:

- Hemoglobin: 8 g% (normal = 12–15 g%)
- Total bilirubin: 5.3 mg% (normal = 0–1.00 mg/dl)
- Conjugated bilirubin: 3.3 mg/dl (normal = 0–0.4 mg/dl)
- Unconjugated bilirubin: 2.0 mg/dl (normal = 0–0.6 mg/dl)
- SGPT: 65 IU/L (normal = 15–45 IU/L)
- SGOT: 148 IU/L (normal = 15–45 IU/L)
- Blood urea: 48 mg/dl (normal = 15–45 mg/dl)
- Serum creatinine: 0.82 mg/dl (normal = 0.6–1.2 mg/dl)



Jaundice with icteric sclera

QUESTIONS

Q.1. Explain the possible reason for such a presentation in this male.

Q.2. What is the biochemical basis of the signs and symptoms?

Q.3. Mention the factors which may precipitate the attack of **acute hemolytic anemia (AHA)** in patient having underlying G6PD deficiency. What is **favism**?

Q.4. Why the deficiency of G6PD is more common in geographical areas where there is high prevalent of malaria?

Q.5. What is the treatment of this disorder?

Q.6. In addition of G6PD deficiency what other enzyme deficiency do you know which may be associated with hemolytic anemia.

Explanations

Ans.1. This patient is suffering with **glucose-6-phosphate dehydrogenase (G6PD) deficiency.** G6PD is protein having 516 amino acid and approximately 140 mutation is known in it. G6PD is the rate-limiting enzyme of HMP shunt pathway which is the major pathway responsible for production of NADPH. This results in inadequate amount of NADPH production in HMP shunt pathway.

Most of the cases are due to **sporadic mutations*** and in inherited conditions, it is inherited as **X-linked disease.** The *G6PD* gene is located on the long arm (q) of the X chromosome (Xq28).

Ans.2. Intake of **primaquine** (an antimalarial drug) has precipitated an attack of hemolytic anemia in this patient who is presenting with breathlessness and dark colored urine.

This patient has underlying deficiency of glucose-6-phosphate dehydrogenase which has compromised the production of NADPH. Deficiency of NADPH does not maintain **glutathione in reduced state** which is necessary to protect the cell's hemoglobin and is important to maintain the membrane integrity. This protects the RBC against highly reactive oxygen radicals **(oxidative stress)***.

Ans.3. Precipitating factors for **acute hemolytic anemia** (AHA) in G6PD deficiency are:

a. Drugs
b. **Fava beans**
c. Certain infections.

Drugs

Hemolytic anemia episodes can result from exposure to certain drugs like **antimalarial, antipyretics and sulfa drugs.** Detail lists of drugs responsible for precipitation of acute episode of hemolysis in patient having G6PD are as follows: Primaquine, Sulfacetamide, Norfloxacin, Acetanilid, Cotrimoxazole, Dapsone, Doxorubicin, Furazolidone, Methylene blue, Moxifloxacin, Nalidixic acid, Naphthalene, Niridazole, Nitrofurantoin, Pamaquine, Pentaquine, Phenazopyridine, Phenylhydrazine, Rasburicase, Sulfanilamide, Sulfapyridine, Thiazolesulfone, Toluidine Blue.

Fig. 2: Primaquine tablets precipitate hemolysis in G6PD deficiency

Fava Beans

Acute hemolytic anemia in G6PD-deficient people can develop after eating fava beans. This is known as **favism.** The chemicals, known as **vicine and convicine**, found within fava beans that trigger acute hemolytic anemia episodes in G6PD-deficient people (Fig. 3).

Fig. 3: Fava beans

Typical Presentation of a Child having Hemolytic Episode

The symptoms begin 2 to 3 days after drug intake or infection but the time of onset of symptom is lesser in **favism.**

A child may have a slightly elevated temperature within 24–48 hours and can become irritable and unruly, or subdued and lethargic*. Nausea, abdominal pain and diarrhea may develop. Urine may become noticeably dark and can appear red, brown or even black. Affected children may become pale and their resting heart rate may be high **(tachycardia)*.** Jaundice can also develop and the liver and spleen may become enlarged.

Ans.4. G6PD deficiency affects individuals of all races and ethnic backgrounds. Approximately 400 million people worldwide suffer from G6PD deficiency. **The highest prevalence rates are found in Africa, the Middle East, certain parts of the Mediterranean, and certain areas in Asia.**

The geographic distribution of G6PD deficiency correlates strongly with the distribution of malaria. It is proved in research that *G6PD* gene mutation conveys **protection from malaria** in these regions. The specific manner how **G6PD deficiency protects against malaria is** not fully understood but possibility is that this protective quality is linked to the inability of malaria to grow efficiently in G6PD-deficient cells.

Ans.5. Treatment is symptomatic and care to be taken to avoids precipitating factors. Before prescribing any of the drug enlisted above (in answer 3) drug history need to be explored and any previous episode of hemolysis need to be ruled out.

Ans.6. Enzyme deficiency responsible for hemolytic anemai. Most common **enzyme deficiency associated with hemolytic anemia** is **G6PD deficiency** which is responsible for 96% cases of enzyme deficient hemolytic anemia. Less than 4% cases are due to deficiency of **pyruvate kinase** and less than 1% cases of enzyme deficient hemolytic anemia are due to **phosphoglucomutase** deficiency.

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Word Meaning

Chills and rigors: Feeling of cold and shivering.

Sporadic mutations: Mutation which is not inherited from parents, rather is acquired.

Tachycardia: Rapid heart rate.

Palpable: Which can be felt by hand.

Oxidative stress: State of excess free radical/reactive oxygen species in the system.

Lethargic: Feeling of weak and sluggish, not interested in doing anything.

CASE

3

Hereditary Fructose Intolerance

A 3-year-old boy is brought in an unconscious state. He had headache, dizziness and a history of intake of **sugarcane juice** followed by weakness. On examination, hepatomegaly was noticed. Baby was icteric* and knee joint tenderness was observed.

Following are the results of various laboratory investigations:

- Blood glucose: 40 mg/dl (normal random blood glucose = 80–140 mg/dl)
- Serum uric acid: 7.9 mg/dl (normal = 2.5–7.2 mg/dl)
- Blood urea: 87 mg/dl (normal = 15–45 mg/dl)
- Serum creatinine: 1.9 mg/dl (normal = 0.6–1.2 mg/dl)
- Blood lactate was high

QUESTIONS

Q.1. What is the most probable diagnosis in this case?

Q.2. What is the biochemical basis of the signs and symptoms?

Q.3. What is the treatment advised?

Q.4. What other disorder is associated with deranged fructose metabolism?

Explanations

Ans.1. This child is suffering with **hereditary fructose intolerance (HFI)** which is due to deficiency of **aldolase B**.

Hereditary fructose intolerance is an autosomal recessive disorder with a frequency of 1 in 20,000. The gene for human aldolase B has been mapped to chromosome 9q22.3.

A single missense* "mutation, G to C transversion*" is the most common mutation found in this disease.

Ans.2. Biochemical basis of signs and symptoms

Hepatomegaly

Aldolase B is the enzyme which is responsible for splitting **fructose-1-phosphate** into **dihydroxyacetone phosphate and glyceraldehyde.**

Out of three isoenzymes of aldolase in the liver, aldolase A, B and C, aldolase B is predominantly found in the liver.

In deficiency of aldolase B, fructose metabolism is affected and fructose-1-phosphate is not further metabolized. Deposition of fructose-1-phosphate results in hepatomegaly.

In addition, sequestration of Pi in the form of fructose-1-phosphate results in absolute deficiency of Pi in the cell which hampers production of ATP by oxidative phosphorylation. Reduced ATP affects ATP-dependent pumps which otherwise are responsible for maintaining the ionic gradient. This results in osmotic lysis of cells.

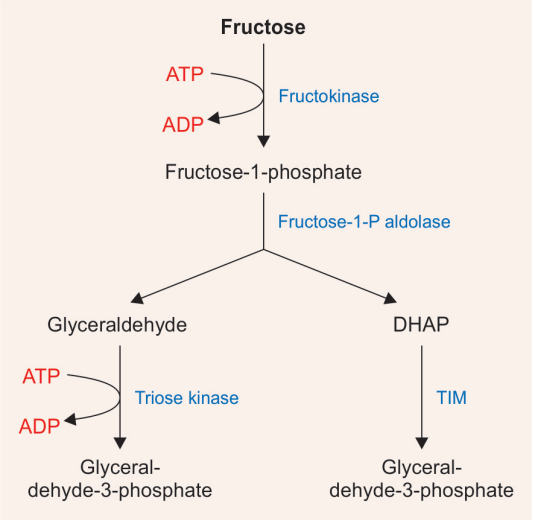

Fig. 1: Fructose metabolism in liver

Hypoglycemia

Glycogen phosphorylase is the rate limiting enzyme of glycogenolysis which provides glucose to blood during hypoglycemia. Fructose-1-PO_4 is the allosteric inhibitor of glycogen phosphorylase enzyme. Glycogenolysis is affected due to inhibition of glycogen phosphorylase by fructose-1-phosphate, resulting in hypoglycemic episode.

Lactic Acidosis

ATP is utilized in converting fructose to fructose-1-phosphate. This results in acute shortage of ATP which increases glycolysis. Increased glycolysis in limited oxygen supply in liver results in lactic acidosis **(Crabtree effect)***.

Hyperuricemia

ATP is a purine nucleotide and it imparts inhibitory affect on purine nucleotide biosynthesis by inhibiting **PRPP synthetase** enzyme.

Increased purine nucleotide biosynthesis (due to sequestration of ATP in converting fructose to fructose-1-phosphate) and then its degradation results in hyperuricemia. Impaired excretion of uric acid in lactic acidosis is also responsible for hyperuricemia. Many of such babies develop kidney failure.

Ans.3. Treatment of such a baby is complete removal of sucrose, fructose and sorbitol from diet which is certainly very difficult to implement because most fruits, vegetables and medicinal preparation contain fructose. Prognosis is poor in this condition and baby dies because of liver and kidney disorders.

Note: In a few case reports, **bilateral cataract*** has been reported in babies having **hereditary fructose intolerance (HFI)**. This is said to be due to inhibitory effect of fructose on sorbitol dehydrogenase which results in excess accumulation of sorbitol in lens cell. **Sorbitol** being a hygroscopic alcohol absorbs water and results in osmotic lysis of cell and initiation of cataract formation.

Ans.4. Other than hereditary fructose intolerance which is due to deficiency of aldolase B, there occurs **deficiency of fructokinase enzyme** which results in disorder known as **benign fructosuria or essential fructosuria**.

As the name itself implies, benign fructosuria is a condition which is a totally benign condition and not harmful. Affected children grow normally. It is detected accidently

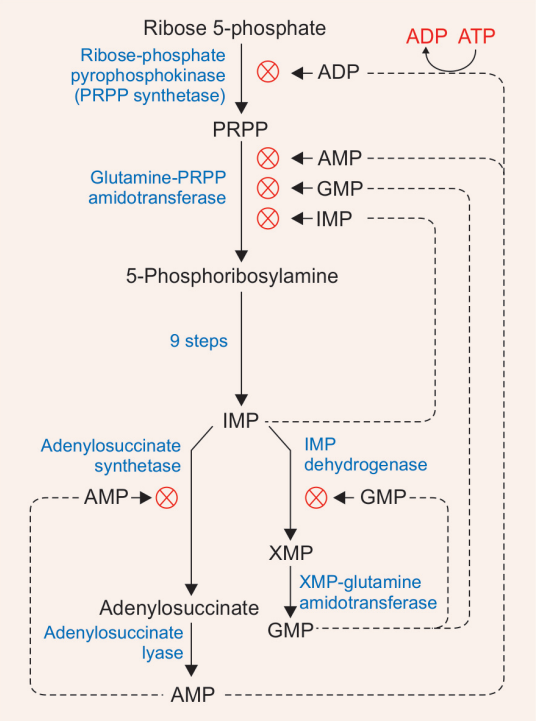

Fig. 2: Regulation of purine nucleotide biosynthesis. ATP is required for supply of ADP which inhibits action of PRPP synthetase

when urine shows positive Benedict test (due to excretion of fructose which is a reducing sugar) in absence of diabetes.

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Word Meaning

Icteric: Yellowish discoloration of skin and sclera.

Missense mutation: A subtype of point mutation where change of nucleotide results in occurrence of a new codon which codes for amino acid which is different from original amino acid.

Transversion: A type of point mutation where purine nucleotide is changed for pyrimidine nucleotide and *vice versa*.

Crabtree effect: Relative anaerobiosis seen in excess glycolysis in limited oxygen supply.

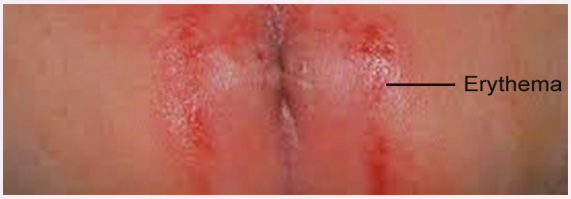
Cataract: Opacity in the lens of eye/s.

CASE

4

Lactose Intolerance

A 1-year-old boy presents to hospital with complain of swelling abdomen, pain and diarrhea after taking milk and other dairy products. These symptoms were existing for past 4 months and started soon after weaning was introduced. Baby is totally alright when fruit juice is given. On examination, perianal skin showed erythema* which may be due to frequent passage of stool with low pH.



Perianal skin showing erythema due to frequent stool of low pH

QUESTIONS

Q.1. What is the most probable diagnosis in this case?
Q.2. What is the enzyme deficiency in this case?
Q.3. What is the biochemical basis of signs and symptoms in this disease?
Q.4. How to diagnose a case of lactose intolerance in a baby?
Q.5. What is the dietary advice given to mother?
Q.6. What are other clinical variants of this disorder? What is adult hypolactasia?

Explanations

Ans.1. Consumption of dairy products precipitate symptoms while on intake of fruit juice, baby is alright. This history suggests that boy is suffering with **lactose intolerance.**

Ans.2. This is due to deficiency of **lactase** enzyme (which is **beta galactosidase**). The gene of enzyme lactase is located on the long arm of chromosome 2 (region 2q21). Maximum lactase expression in intestinal cell occurs during the first months of life and declines after weaning.

Deficiency of this enzyme is common in preterm infant. This is the last **disaccharidase** which develops during intrauterine development, hence the deficiency of this enzyme is commonly seen in babies born prematurely.

The lactase enzyme is often situated in the microvilli of the small intestine (specially mid-jejunum) and it hydrolyses dietary lactose into its monomer D-glucose and D-galactose which are then being absorbed.

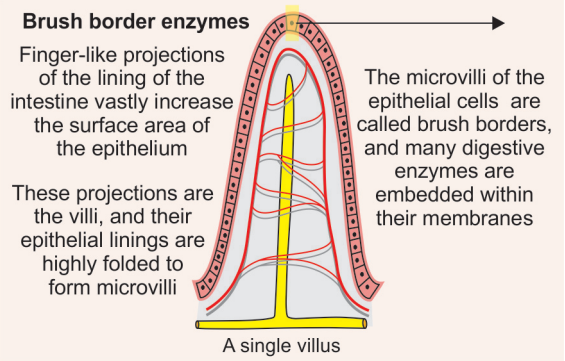


Fig. 1: Lactase is a brush border enzyme

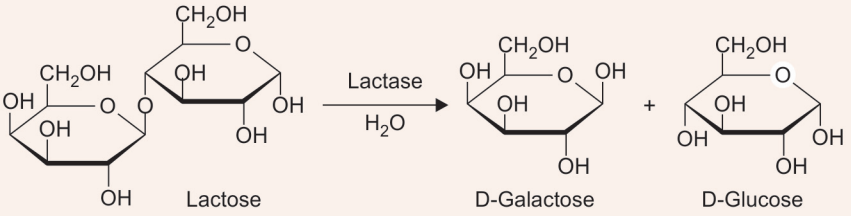


Fig. 2: Action of lactase on lactose

The enzyme spans the apical membrane of mature enterocytes and is homodimer (made up of two identical extracellular 160 kDa polypeptide chains) as well as a short intracytoplasmic part.

In many populations, **lactase levels** decline after weaning. This condition is called **lactase non-persistence (LNP).** LNP affects approximately 70% of the world's population and is the underlying physiological factor for primary lactose intolerance (LI).

While the decline in lactase levels starts soon after weaning, symptoms generally do not manifest before 5 years of age. Presentation in earlier age is most commonly associated with underlying gut condition like viral gastroenteritis, cow milk allergy, giardiasis, coelic disease, etc.

Ans.3.

BIOCHEMICAL BASIS OF SIGNS AND SYMPTOMS IN A CASE OF LACTOSE INTOLERANCE

Abdominal Distension and Bloating

The undigested and unabsorbed lactose in the lumen is osmotically active and tends to absorb fluid, resulting in diarrhea. It also becomes a substrate for action of intestinal bacteria which ferment the lactose in the colon to short-chain fatty acids (SCFA), hydrogen (H_2), carbon dioxide (CO_2) and methane (CH_4).

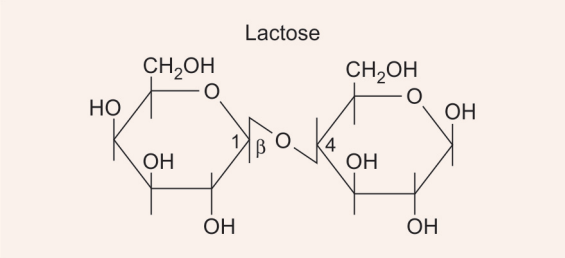


Fig. 3: Lactose is a disaccharide

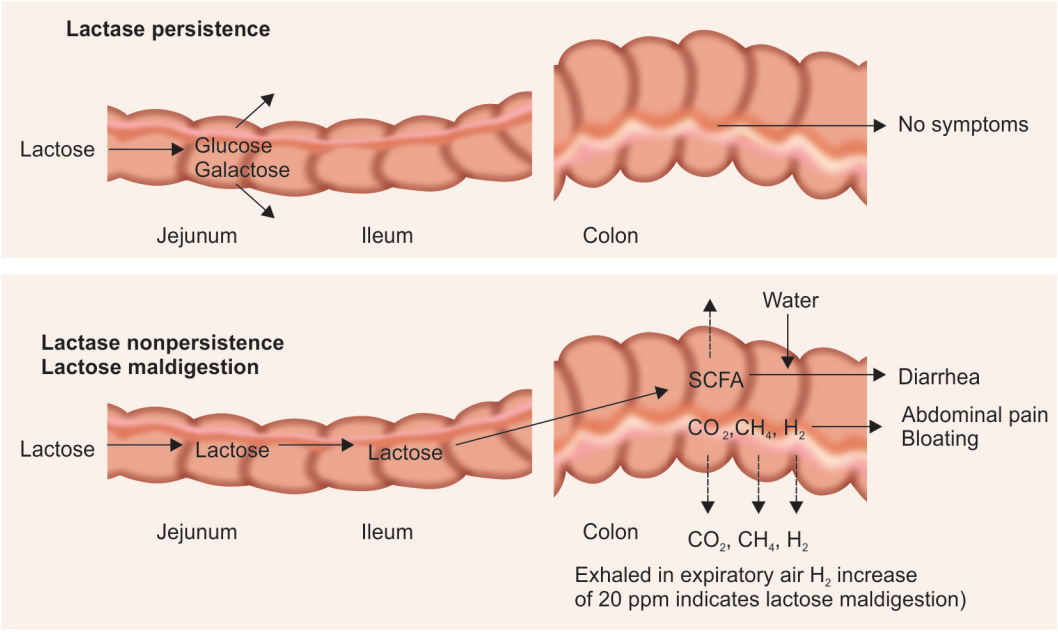


Fig. 4: Mechanism involved in abdominal pain, bloating in lactase nonpersistence (LNP)

These gases are responsible for flatulence, bloating and distension in the abdomen after consumption of milk and dairy product.

Fig. 5: Lactose-containing food items

Ans.4. Diagnosis may be done based on the following findings:

a. The **stool pH** in infants with lactose intolerance is typically **below 5.5 to 6.0**.
b. Measurement of **total and reducing sugars in stool** is an indirect test for lactose malabsorption.
c. **Breath hydrogen test:** In this test, exhaled hydrogen is measured after giving a standard dose of lactose. After an overnight fasting period, baseline hydrogen should be closed to 0 parts per million (ppm). Breath samples are taken every 30 min for 3 hr (from time of the lactose bolus).

   A rise in exhaled hydrogen by 0.20 ppm from baseline is considered diagnostic of lactose intolerance.

Ans.5. Mother is advised to give lactose- and galactose-free diet to this baby till the symptoms subside. Thereafter lactose-containing foods should be reduced but do not need to be eliminated completely.

Breast milk has high content of lactose (7.5 g/100 ml) but it should be continued.

Following are the food items having different amount of lactose:

Food	*Lactose content (g) per 100 g*
Milk (skimmed)	4.8
Milk (full)	4.7
Yoghurt (fresh)	3.0
Buttermilk	3.0
Cheese	3.0
Butter	0.5

Ans.6. Adult-type hypolactasia is characterized by the downregulation of the lactase enzyme activity in the intestinal wall with the advancement of age. Lactose intolerance is generally reversible while adult hypolactasia is an irreversible condition.

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Word Meaning

Erythema: Redness of the skin.