28

◉ Sheet ○ Slides

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Amino acid metabolism: Disposal of Nitrogen

Amino acids are organic compounds, have an alpha-carbon which is connected to Carboxyl group, amino group, hydrogen atom and R group which differs from an amino acid to another. Except for glycine that connect to hydrogen atom instead of R group.

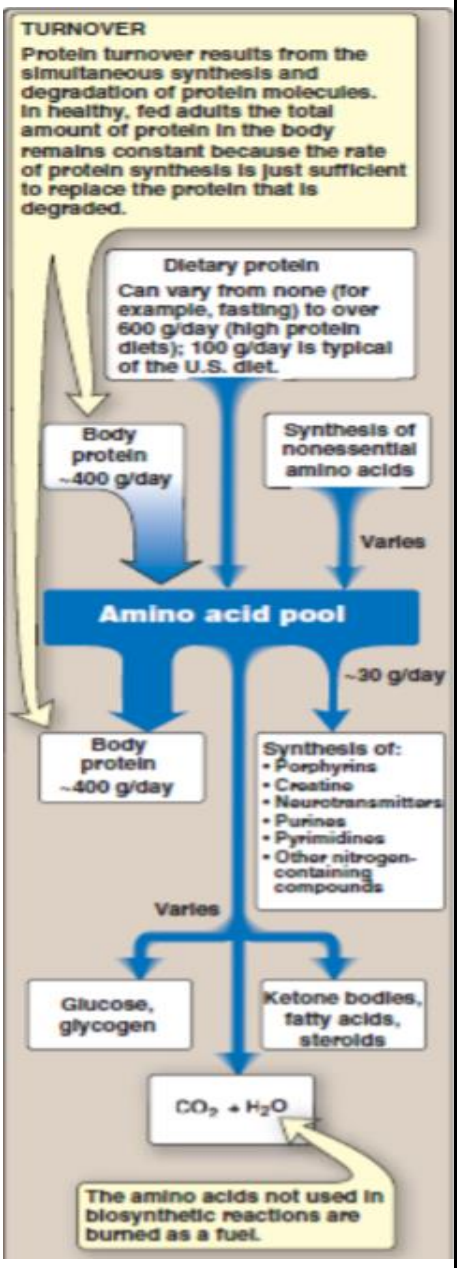
The AA pool

The AA pool is small ~about 90–100 g of AAs, because the amino acids can't be stored.

Normally, the amount of amino acids in the AA pool is balanced, so at all times we have input (sources of AA) accompanied with output (depletion routes)=>(constant amount).

The amount of protein in the body is about 12 kg in a 70-kg man.

• The amino acid pool is in a steady state, and the individual is in **nitrogen balance**.



AAs pool sources:

1- Diet: Exogenous protein digestion.
2- De novo synthesis: we can't synthesize all the 20 amino acids, we only synthesize some of them that can be categorized as nonessential amino acids. The essential amino acids can't be synthesized so we have to obtain them from diet.
3- Protein degradation: Endogenous protein ➔ whether misfolded proteins or proteins that have done their function or after they bind to a permanent inhibitor irreversibly.

Amino Acid Pool Depletion Routes:

1) Synthesis of body protein.
2) AAs consumed as precursors of nitrogen containing small molecules (hormones, pigments) without the removal of amino group.
3) Synthesis of other molecules ➔glucose, glycogen, fatty acids, ketone bodies, or *CO2 + H2O*.

** The amino acids can used as a source of energy by converting them into CO2.*

AA metabolism overview:

We have to maintain the **nitrogen balance**; why?

Because nitrogen as atom contributes to the synthesis of ammonia (highly toxic compound) so we need to get rid of this molecule by converting it into less toxic compound → **UREA**, then it gets excreted with urine.

1- AT FIRST: α-amino group is removed by ***transamination*** (transfer the **amino group** from **amino acids** to **α-keto acids**) **so the amino acids are converted to α-keto acids (carbon skeleton)**

Once the α- keto acid receives the amino group it becomes amino acid.

So, Why do we do this process ?

In this process we convert all 20 amino acids types into one type of amino acid; so in the next step there is only one enzyme specific to this type of amino acid. (will be explained later)

2- THEN: oxidative deamination→ the amino group is removed in the form of ammonia that is toxic, so the ammonia enters the urea cycle, that happens in hepatocyte to convert ammonia to urea then it gets excreted with urine.
Leftover α-keto acids are converted to energy producing intermediates that can be metabolized to CO2 , water, glucose, fatty acids, or ketone bodies.

the dilution of toxic compounds makes them less toxic; so why they do not get diluted?

It's unpractical, because to dilute ammonia to nontoxic levels, we need very large amount of liquid that's not present in our body.

The body removes the amino group when it does not need to synthesize nitrogen containing compounds
The metabolic processes have to keep harmony between **amino acid pool** and **protein turn over.**

Protein turn over

Protein turnover is the process in which the rate of protein synthesis is sufficient to replace the degraded protein, that keeps the total amount of protein constant.

Each day, 300–400 g of body protein is hydrolyzed and resynthesized

Rate of turnover varies widely for individual proteins depending on their half-life and their function.

- Structural proteins, such as collagen, are metabolically stable (t1/2 months or years).
- Most proteins are long-lived proteins (t1/2 days to weeks)
- Short-lived proteins such as many regulatory proteins and misfolded proteins, are rapidly degraded, t1/2 is min-hours.

What affects the half life of a protein?

- **The nature of the N-terminal residue**: Some scientists connected some structural features with the half-life of proteins.

✓ Proteins with Ser at the N-terminus are relatively long-lived (t1/2 is > 20 hrs).
✓ Proteins with Asp at the N-terminus are very short-lived (t1/2 of only 3 minutes).
✓ Proteins rich in sequences containing Pro, Glu, Ser, and Thr (PEST sequences) are rapidly degraded (short t1/2).

That's not for memorizing ➔ just to show you how structural aspects are related to the half-life of the protein.

- **Mode of expression:** when the protein is synthesized.

a. Many proteins have induced expression mode, which need certain stimuli to be synthesized, e.g: enzymes. Regulation of synthesis determines the concentration of protein in the cell, with protein degradation assuming a minor role.

b. Other proteins have constitutive expression mode, that the rate of synthesis is relatively constant, and cellular levels of the protein are controlled by selective degradation. E.g: House-keeping genes

- The proteins that are expressed by induction have short half-life; because they do specific function at specific case and get degraded. Once we need them more, we need to stimulate the expression of these molecules again.

protein degradation

The protein degradation happens internally (inside the cell)

In case of **Misfolded protein**: the chaperone makes them unfolded and try to fold them again.

➔ if they pass, its fine ☺
➔ If they don't, they get tag (targeted) by ubiquitin in order to be recognized for degradation ☹

So the proteins that have done their functions as well as the misfolded proteins are targeted for degradation.

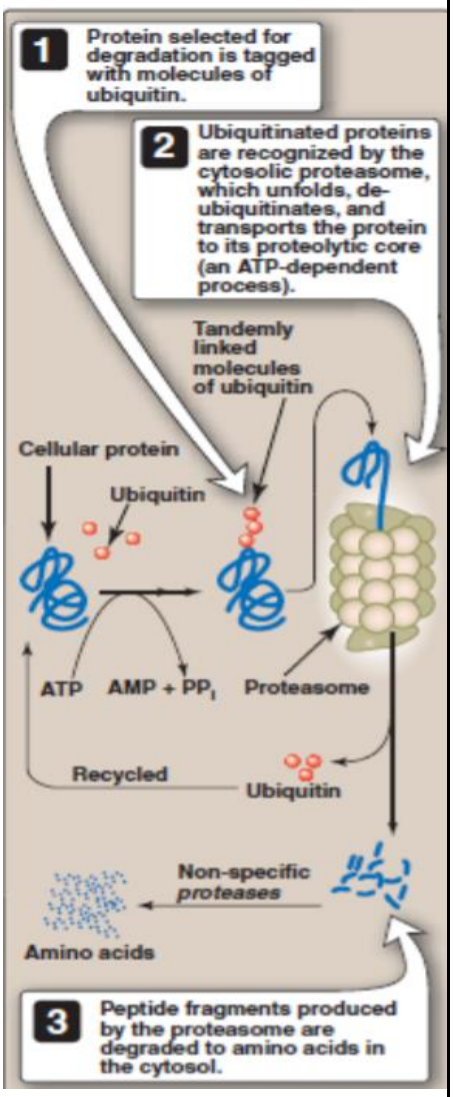
How are proteins tagged for degradation? By chemical alterations such as oxidation or ubiquitin tagging.

There are **two major enzyme systems** are responsible for degrading damaged or unneeded proteins:

- ***Lysosomal system:*** The **ATP-independent** degradative enzyme system of the lysosome enzymes (acid hydrolases) degrade primarily:
  - Extracellular proteins, such as plasma proteins, by endocytosis.
  - Cell-surface membrane proteins by receptor-mediated endocytosis.

These proteins will enter the cell forming an endosome, then this endosome fuses with the lysosome and they get exposed to the lysosomal enzymes (digestive enzymes that act under low PH), so the proteins get denatured and digested into amino acids.

- ***ubiquitin-proteasome system:*** The **ATP-dependent** ubiquitin-proteasome system of the cytosol mainly endogenous proteins (proteins that were synthesized within the cell).



A. ***Proteins selected for degradation by the ubiquitin-proteasome system are first covalently attached to multiple molecules of ubiquitin(a small, globular, non-enzymatic protein).***

B. ***Ubiquitination of the target substrate is linking the α-carboxyl group of the C-terminal Gly of ubiquitin to the ε-amino group of a Lys on the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process.***

> ***Several Ub units are added to generate a polyubiquitin chain.***

C. ***Proteins tagged with ubiquitin are then recognized by the proteasome (a large, barrel shaped, macromolecular, proteolytic complex***)

A proteasome: is a multi-protein complex that forms a barrel shaped structure which is hollow from the inside (not an organelle)

D. ***The proteasome unfolds, deubiquitinates and cuts the target protein into fragments that are then further hydrolyzed by nonspecific proteases into amino acids.***

- The ubiquitin isn't needed to act as hydrolytic enzyme, its needed for the adding (polyubiquitination process).
- Ubiquitination is the process that needs ATP.

  Ubiquitin does not get digested, instead it is recycled again to tag another protein.

  Simple hydrolysis by nonspecific proteases does not require energy

Clinical Application: HPV and cervical carcinoma

In most cases cervical carcinoma are caused by Human Papillomavirus (mainly strain 16 and 18), that are present normally in some males and does not cause any diseases or signs, then by sexual relationships this virus is transferred into female and causes infection in cervical cells.

HPV enters the cell with its genetic materials and starts to use the machinery of the cell and induce gene expression of many genes, one of these genes is the gene that encodes for E3 enzyme which increases the ubiquitination of P53 protein (P53 gene is a tumor suppressor gene), so once P53 is ubiquitinated it is going to be degraded and it loses the function of tumor suppressor gene, so the over activation of cell cycle happens ➔ which leads to conversion of cervical cells into cancerous cells.

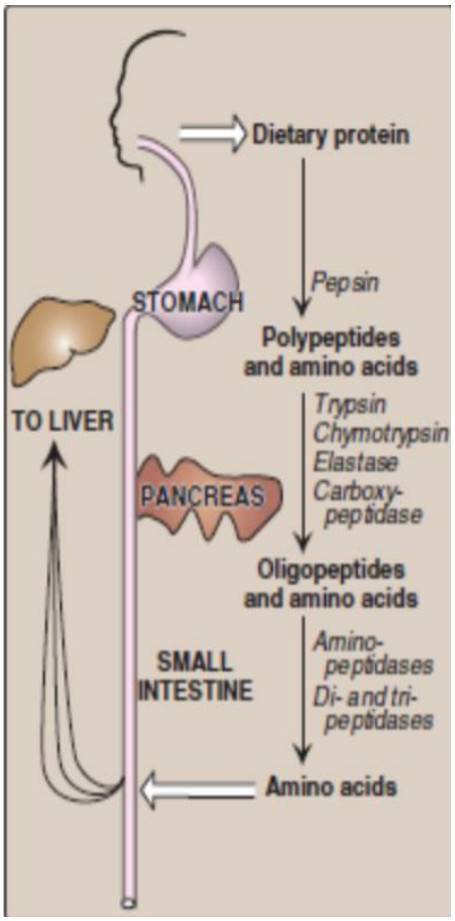
The digestion of exogenous (dietary) proteins

Digestion of proteins by gastric secretion

Proteins are too large to be absorbed by the intestine.
Protein digestion begins in the stomach not in the oral cavity due to their complexity and large size.
Stomach secretes the gastric juice that contains hydrochloric acid and the proenzyme, pepsinogen.

1- ***Hydrochloric acid***: secreted by the **parietal cells** (the cells that control PH value), pH 2–3 to hydrolyze proteins.

HCl functions: A. kills some bacteria



B. denatures proteins to make them more susceptible to subsequent hydrolysis by proteases.

2-pepsin: acid-stable endopeptidase, secreted by the **chief cells** of the stomach as an inactive zymogen (or proenzyme), pepsinogen.

-Pepsinogen is activated to pepsin, either by HCl, or autocatalytically by other activated pepsin molecules.

The zymogens are activated by cleavage in the N terminus which open the active site, so it can hydrolyze peptide bonds

*Pepsin releases **polypeptides** and a few free amino acids from dietary proteins, so in order to complete the digestion these polypeptides enter (along with other food materials and Stomach juice and pepsin in a form of mixture called chyme) the small intestine, and the inactivation of the pepsin takes place due to the increase in the PH in the intestine.*

Digestion of proteins by pancreatic enzymes in small intestine

Group of pancreatic enzymes (proteases) released into the duodenum including → trypsin, chymotrypsin, elastase, carboxypeptidase A&B.

All of these proteins released as zymogens (trypsinogen, chymotrypsinogen, pro-elastase, pro-carboxypeptidase A&B). and once they reach the intestine they become activated and they start digesting these polypeptides.

Release of zymogens: *The release and activation of the pancreatic zymogens is mediated by the secretion of cholecystokinin and secretin (two polypeptide hormones of the GIT)*

Zymogen activation: *Enteropeptidase (enterokinase)— the luminal surface of intestinal mucosal cells converts the pancreatic zymogen trypsinogen to trypsin (removal of a hexapeptide from the N-terminus of trypsinogen)*

Trypsin subsequently converts other trypsinogen molecules to trypsin

Trypsin is the common activator of all pancreatic zymogens

*So the **large polypeptides** produced in the stomach are further cleaved to **oligopeptides** and amino acids by these proteases.*

You may wonder, why do we need 5 types of proteases?

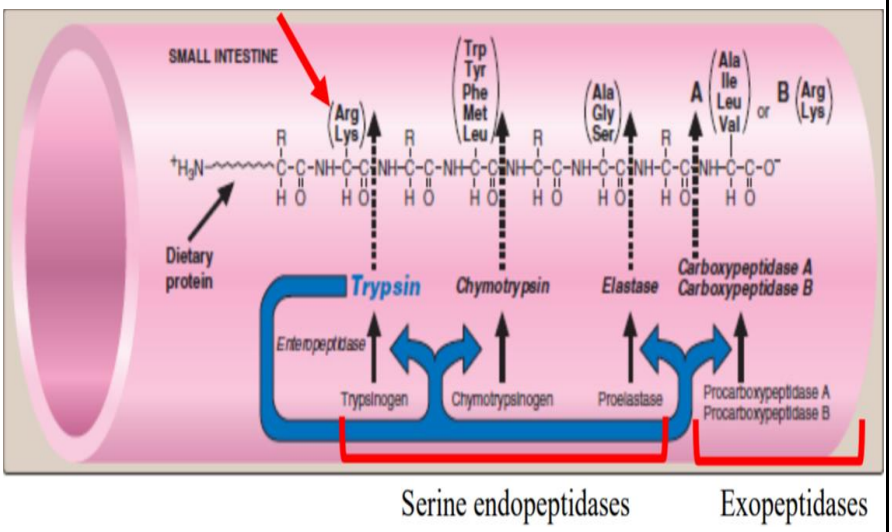
The answer is: each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond.

Trypsin breaks **after** Arg &Lys.

Chymotrypsin breaks **after** Trp, Tyr, Phe, Met & Lue.

Elastase breaks **after** Ala, Gly & Ser.

So the trypsin, chymotrypsin, elastase as well as pepsin are **endopeptidases** (break peptide bonds of nonterminal amino acids within the molecule).



carboxypeptidase A&B breaks **before** Ala, Lys, Arg …, it hydrolyzes amide bond at the carboxyl or C-terminal end of peptides so they are classified as **exopeptidases**.
So we hydrolyzed the protein into polypeptides then polypeptides into oligopeptides by endopeptidases and exopeptidases that break the bond at C- terminal end, what about the N-terminal end?

There is an specific enzyme for the N-terminal end, but it's not a pancreatic enzyme it's an intestinal enzyme

Digestion of oligopeptides by enzymes of the small intestine

Aminopeptidase at the luminal surface of the intestine.

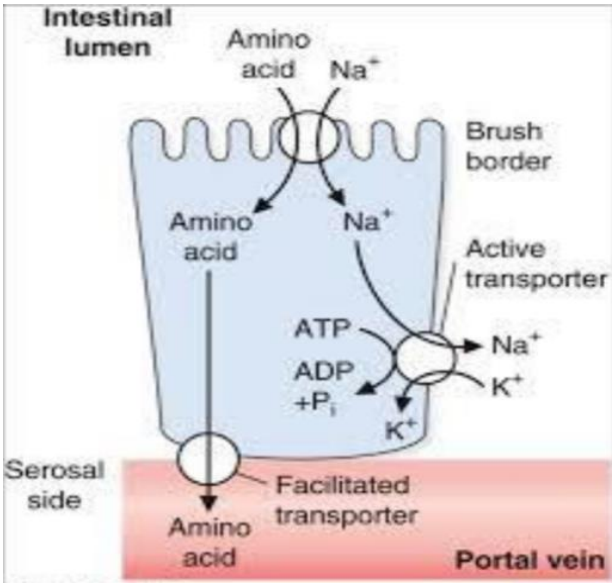
*Aminopeptidase is an exopeptidase that repeatedly cleaves the N-terminal residue from **oligopeptides** to produce **smaller peptides (di- or tri-peptides)** and free AAs.*

Absorption of amino acids and small peptides

Even if certain amino acid was nonpolar, it can't cross cell membrane; WHY?
It has an amino group and a carboxyl group on its backbone so the nonpolar chain on its R-group can't influence its ability to cross the membrane.
Since the amino acids have at least one polar side and they have large size, this prevent them from crossing the membrane. So we need transporter system.
Free amino acids are taken into the enterocytes by two mechanisms depending on amino acid type :

- Ω Na+-independent transport.
- Ω Na+-linked secondary transport system of the apical membrane.

Di- and tri-peptides *are absorbed by a H+-linked transport system and hydrolyzed in the cytosol to* ***amino acids****.*



AAs are released into the portal system by facilitated diffusion (because the concentration of the amino acids becomes high inside the intestinal cytosol) by different types of transport systems in the basolateral surface. Then they go from the portal circulation to the liver for metabolism, or they get released into the general circulation.

Branched-chain amino acids are not metabolized by the liver, but are sent from the liver to muscle via the blood.

Clinical Hint: Abnormalities in protein digestion and Celiac disease

Pancreatic secretion deficiency due to chronic pancreatitis, **cystic fibrosis**, surgical removal of the pancreas or pancreatic cancer, results in incomplete fat and protein digestion (no amylases, no lipases, no proteases) resulting in malabsorption and the excretion of proteins, lipids and complex sugars through feces.

Symptoms: abnormal appearance of lipids (steatorrhea), and undigested protein in the feces.

Cystic fibrosis: is a genetic disease, defect CFTR protein impacts the function of the pancreas and alters the consistency of mucosal secretions → the pancreatic exocrine secretions have to move within the duct and go to their site of action, but with this disease these secretions become thick, that may clog the duct.

Celiac disease (celiac sprue) is a disease of **malabsorption** resulting from immune-mediated damage (allergic reactions) to the small intestinal cells specifically brush border leading to less efficient absorption in response to ingestion of gluten (or gliadin produced from gluten), a protein found in wheat, barley and rye that work as an allergen. → This disease is treated with gluten free diet.

After the amino acids are absorbed and move to the portal circulation they need a transport system to transfer them into the cells.

transport of amino acids into cells

[Free AAs in the extracellular fluids] <<< [Free AAs within the cells]

Different transport systems have overlapping specificities for different AAs

These transport systems may be exclusive (for one amino acid type) or may be for different amino acid types

AA transport systems:

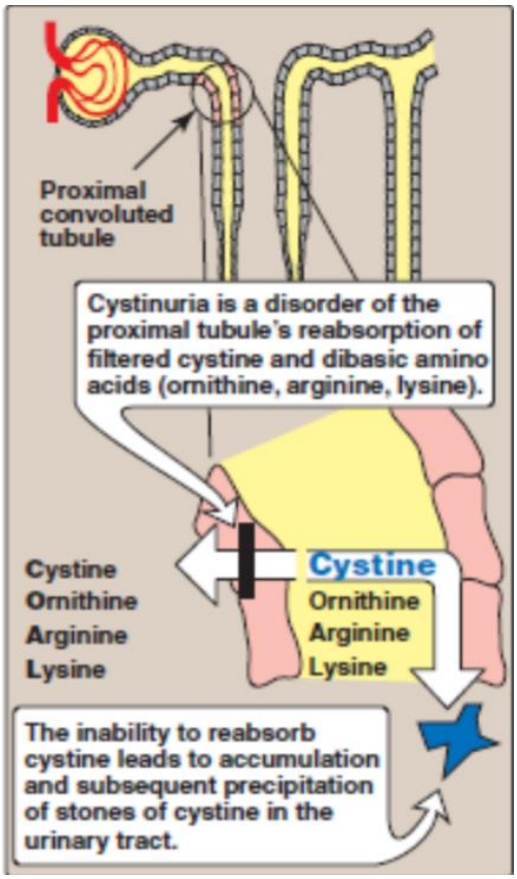
The small intestine and the proximal tubule of the kidney

One system uptakes **cystine** (two cysteine molecules joined together by Disulfide Bridge) and the dibasic amino acids, ornithine, arginine, and lysine (represented as "COAL").

{Ornithine is an amino acid that is found in our body and contributes in some metabolic processes. It's not found in proteins.}

Defects in the transport of tryptophan (and other neutral amino acids) results in Hartnup disorder and pellagra-like dermatologic and neurologic symptoms.

Clinical Hint: Cystinuria



Rare Inherited disorder, is the most common genetic error of amino acid transport.

The COAL carrier system is defective, and all four amino acids appear in the urine.

The amino acids go into tubular fluid (the fluid that will be converted to urine later on) and the body reabsorb them in order to reuse them again.

But because of this genetic problem in this transporter system the body can't reabsorb these amino acids and their concentration will become high in this fluid and they reach the oversaturation state and starts to precipitate in this fluid forming kidney stones (calculi) that can be microscopic and macroscopic. the microscopic are more dangerous than the macroscopic due to their ability to move into variety of places.

Oral hydration is important for the treatment of this disorder.

Some questions to test yourself →

1- True sentence about amino acid digestion:-

A-monopeptides are absorbed by passive diffusion

B- monopeptides enter the portal system by NA+ linked transport

C- Di-Peptides are taken up by H+-Linked transport system

2- A 7-month-old infant is brought to the physician's office because of poor weight gain despite large food intake. He has had two episodes of pneumonia and has frequent bulky stools. He coughs frequently. X-rays of the lungs show increased markings and hyperinflation. Trypsin is absent in a fresh stool sample, and the fat content is increased. Which of the following is the most likely cause of this infant's disorder?

A. Autoimmune disorder
B. Defective ion transport at epithelial surfaces
C. Disaccharidase deficiency
D. Inability to synthesize apolipoprotein B
E. Villous atrophy of the jejunum

3- An 11-year-old girl with celiac disease was discharged from the hospital. An appropriate teaching was carried out by the nurse if the parents are aware of avoiding which of the following?
A. Chicken
B. Wheat
C. Milk
D. Rice

ANSWERS:

1-C, 2-B, 3-B

Q1 ➔ The correct answer is c:

Di- and Tri-peptides are absorbed by specific system H+-Linked transport system(choice C), the answer is not A→ mono peptides are not absorbed by passive diffusion due to presence of at least one polar side, and the answer is not B→ monopeptides do not enter the portal system by NA+ linked transport, in contrast they enter the portal system by facilitated diffusion.

Q2 ➔ The correct answer is B:

This infant has the clinical presentation of cystic fibrosis, which is due to defective ion transport at epithelial surfaces (choice B). This is an autosomal recessive disease due to a mutation in the chloride transporter, cystic fibrosis transmembrane conductance regulator (CFTR). Patients present with meconium ileus, deficiencies of pancreatic enzymes, pulmonary obstruction, frequent pulmonary infection, bronchiectasis, cor pulmonale, and respiratory failure. Other findings include liver cirrhosis, infertility, and elevated NaCl concentrations in sweat.
This is not due to an autoimmune disorder (choice A), disaccharidase deficiency (choice C), inability to synthesize apolipoprotein B (choice D), or villous atrophy of the jejunum (choice E).

Q3 ➔The correct answer is B:
B: Children with celiac disease cannot tolerate or digest gluten. Therefore, because of its gluten content, wheat and wheat-containing products must be avoided.
A, C, D: Rice, milk, and chicken do not contain gluten and need not be avoided.

GOOD LUCK