Catabolism of proteins

Seminar No. 5

Amino acid pool

~ 80 % in muscles

~ 10 % in liver

~ 5 % in kidney

~ 5 % in blood

AA pool is not reserve

There is not a specific protein reserve in human body in contrast to saccharides (liver glycogen) and lipids (adip. tissue).

What are three sources and three uses of AA pool?

Overview of AA metabolism

Three sources of AA pool:

1) Proteolysis of food proteins
2) Proteolysis of tissue proteins
3) Synthesis of non-essential AA

Three uses of AA pool:

1) Synthesis of tissue and plasma proteins
2) Synthesis of specialized nitrogen products
3) Deamination + utilisation of carbon skeleton

What are **three** possible uses of AA carbon skeleton?

Q. 1

A. 1

- Stomach – pepsin
- Small intestine: trypsin, chymotrypsin, elastase, carboxypeptidase A/B, aminopeptidase

What kind of reaction do these enzymes catalyze?

Q. 2

A. 2

Hormon	**Stimulates**
Gastrin	the secretion of HCl and pepsin in the stomach
Secretin	the production of pancreatic juice, esp. HCO_3^-
Pancreozyme (cholecystokinine)	the production of pancreatic enzymes, the contraction of gall bladder

Q. 3

A. 3

L-amino acids:

about seven **specific** transporters, symport with Na^+

D-amino acids (trace amounts):

nonspecific diffusion, hydrophilic pores in membranes,

D-AA cannot be utilized in the body,

they are only catabolized to gain energy

What food is the source of D-amino acids?

Q. 4

A. 4

Intracellular proteases degrade endogenous proteins, two systems:

- **Lysosome** (non-specific degradation, no ATP)

  Extracellular + membrane proteins

- **Ubiquitin-proteasome** (ATP needed)

  damaged/misfolded proteins,

  regulations proteins (with short half-life)

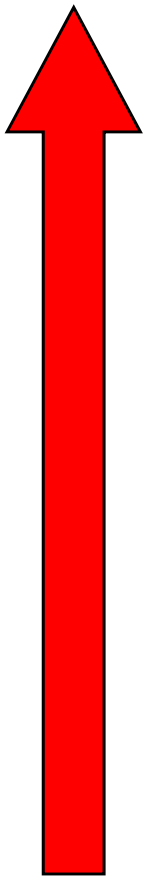
Q. 5

A. 5

Glucogenic (14)	Ala, Arg, Asp, Asn, Cys, Glu, Gln, Gly, His, Met, Pro, Ser, Thr, Val
Ketogenic (1 or 2)	Leu (Lys)
Mixed (5)	Ile, Lys, Phe, Trp, Tyr

Biological value of some proteins

Protein	BV (%)
Egg white	100
Whey	100
Whole egg	96
Casein	80
Beef	80
Pork	70
Oats	60
Wheat flour	54
Beans	49
Gelatine	25



Simplified definition:

the amount of endogenous proteins

(in grams) made in body from 100 g

of dietary proteins

Whey

- a by-product at (cottage) cheese production
- yellowish liquid (the colour comes from riboflavin)
- cca 12 % of high quality proteins (lactoalbumin, lactoglobulins)
- rich in other B-complex vitamins and lactose
- dried whey is available in shops (esp. fitness centres)

Q. 6 + 7

A. 6 + 7

- Valine (branched)
- Leucine (branched)
- Isoleucine (branched)
- Threonine (2 C*)
- Phenylalanine (aromatic ring)
- Tryptophan (aromatic ring)
- Lysine (basic, two NH_2 groups)
- Methionine (S-CH_3)

Conditionally essential aminoacids

histidine, arginine (in childhood and youth)

alanine, glutamine (in metabolic stress)

about 30 % of methionine requirement can be made up by cysteine

about 50 % of phenylalanine requirement can be made up by tyrosine

Q. 8

A. 8

Most plant food

- cereals, rice, corn (maize) – lack of Lys, Trp, Thr, Met
- legumes – lack of Met

Some animal food

- gelatin (lack of Trp)
- game, octopus, lobster (low digestibility)

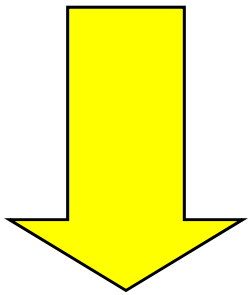
Conversions of AA after meal

- AA from food are absorbed from intestine
- Glutamate +glutamine are utilized as **metabolic fuel** for enterocyte
- 20 % of AA in portal blood are branched AA
- In liver, most AA are utilized for synthesis of proteins, Glc, FA.
- Val, Leu, Ile are not metabolized in liver due to the lack of aminotrasferases $\Rightarrow$ they predominate (70 %) in central circulation
- High content of ammonia in portal blood is removed by liver $\rightarrow$ urea

Q. 10

A. 10

Carbon skeleton of AA is used to make FA and TAG



Highly protein diet invariably leads to obesity

Q. 11

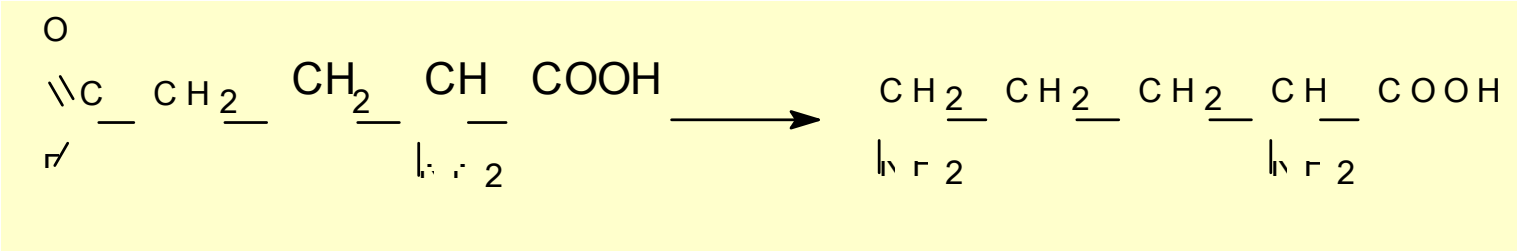
A. 11

Because of lack of specific aminotransferases in liver

Q. 12

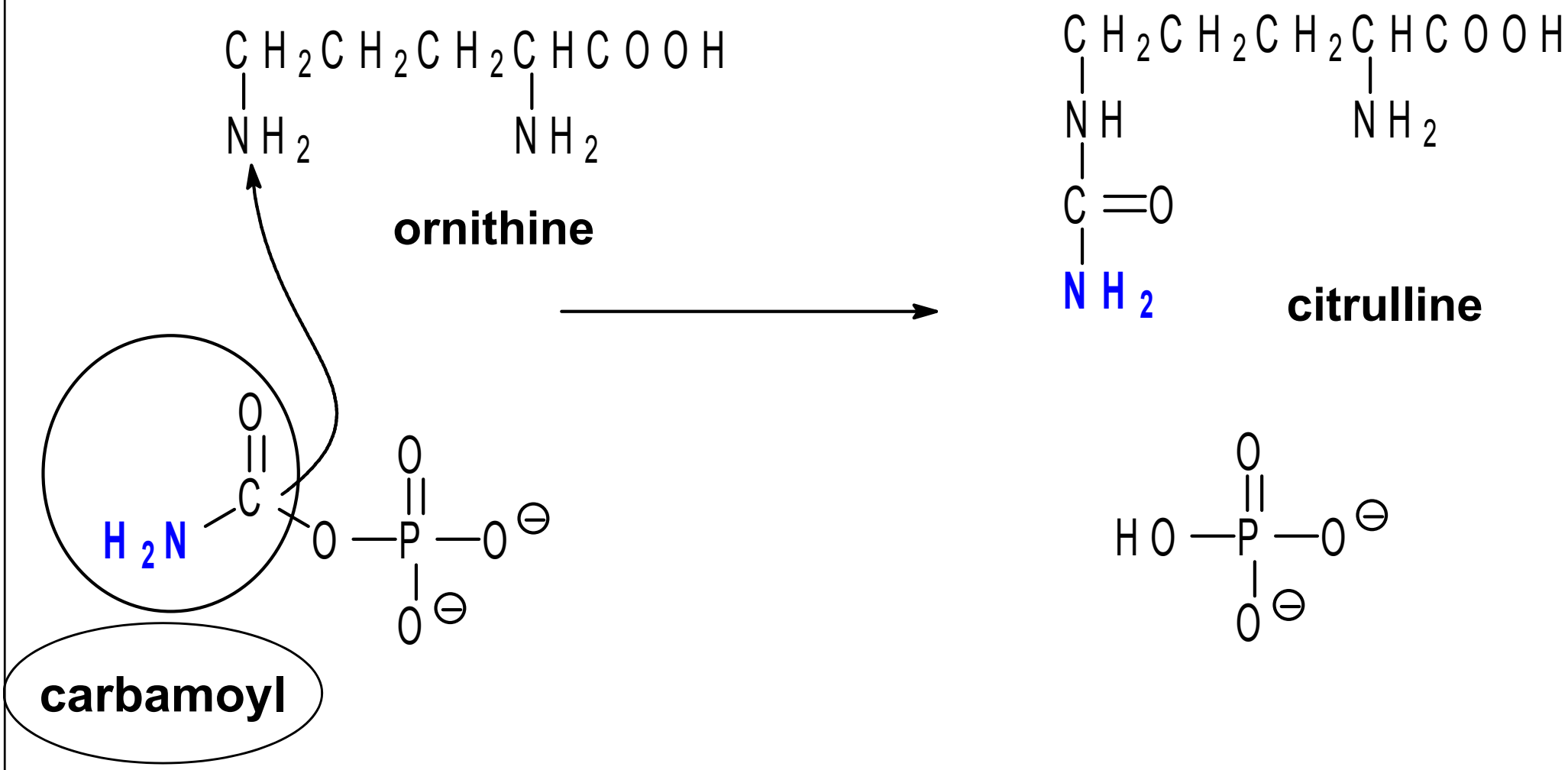
A. 12

- glutamine is deaminated to glutamate
- glutamate + NADPH+H^+ → glutamate semialdehyde + ADP + P_i
- glutamate semialdehyde is transaminated to ornithine



- **ornithine + carbamoyl phosphate → citrulline**
- citrulline is transported to kidneys where it is converted to arginine
- arginine is utilized in liver for urea

Citrulline is made by the addition of carbamoyl group to ornithine



Q. 13

A. 13

1. **Deaminations of glutamine + glutamate** in enterocyte

2. **Bacterial putrefaction of proteins in the large intestine**

   produces nitrogen catabolites (e.g. biogenic amines + ammonia),

   ammonia diffuses freely into portal blood $\Rightarrow$ portal blood has high

   concentration of NH_4^+ $\Rightarrow$ eliminated by liver

How can you decrease the production of ammonia in the human body?

1. **Low-protein diet** (especially important in liver diseases)
2. **Alteration of colon microflora by the ingestion of:**

- **Probiotics** – live bacteria stimulating saccharolytic (fermentative) processes in large intestine instead of putrefactive ones (*Lactobacillus*, *Bifidobacterium*) – yoghurt, kefir milk
- **Prebiotics** – non-digestible food ingredients that stimulate the growth probiotics in the colon (dietary fibre, lactulose, oligofructose, inulin) – e.g. soybeans, Jerusalem artichokes (inulin), chicory root (inulin), oats ...

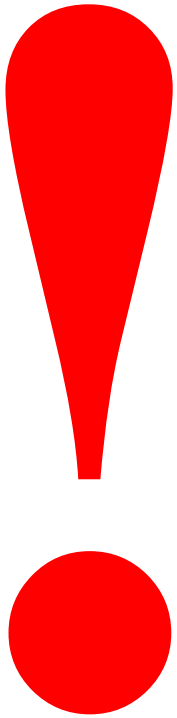
Ammonium ions in body fluids

Body fluid	NH_4^+ (mmol/l)	Metabolic origin of NH_4^+
Urine	**10 – 40**	hydrolysis of Gln, deamination of Glu (tubules)
Saliva	**2 – 3**	hydrolysis of urea by oral microflora
Portal blood	**0.1 – 0.3**	protein putrefaction (GIT), Gln/Glu (enterocyte)
Venous blood	**0.005 – 0.030**	catabolism of AA in tissues

Conversions of AA in fasting

- There is no special protein store in the body
- Liver proteosynthesis is limited, proteolysis in muscles increases (insulin ↓, cortisol ↑)
- The main AA released from muscles are Ala + Gln
- Ala is the substrate of liver gluconeogenesis
- Gln is deaminated in liver to give NH_4^+ - urea synthesis (periportal region)
- Gln is made in perivenous region – the detoxication of remaining ammonia

Q. 19



A. 19 - Gln in muscle

- Gln is released by proteolysis
- Gln is product of ammonia detoxication
- Gln can be viewed as a carrier of $-NH_2$ group from muscles to liver (periportal hepatocytes) where NH_3 is liberated and converted to urea

A. 19 – Gln in enterocyte

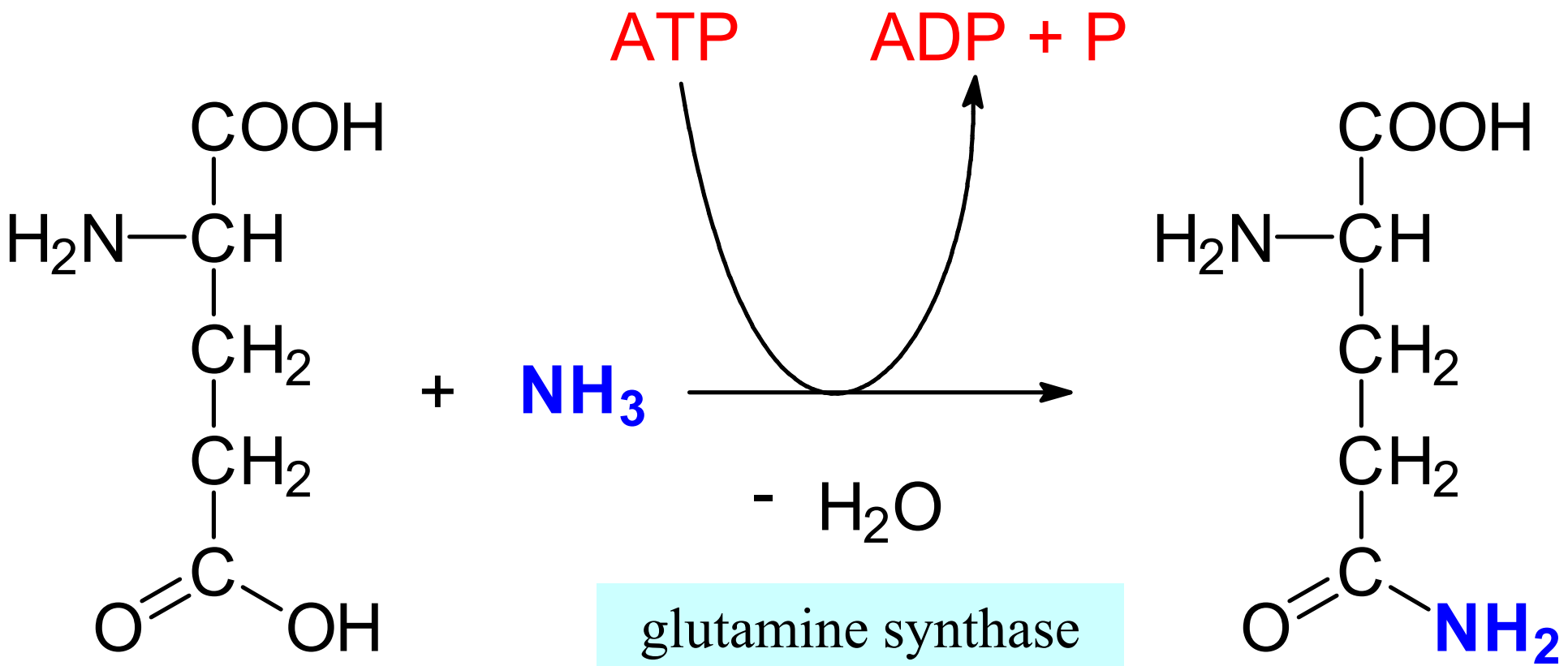
- exogenous and endogenous Gln is the source of energy for intestinal mucosa: Gln → 2-OG → energy (CAC)
- enterocytes have high turnover – Gln (and other AA) are needed for proteosynthesis and nucleic acid bases
- limited usage of glucose and FA as fuel in enterocyte

A. 19 – Gln in brain

- Glutamine formation is the principal way of ammonia detoxication in CNS
- Glutamine synthase reaction occurs mainly in astroglial cells
- In other CNS cells is Gln the source of glumate – as the substrate for GABA

How is GABA made from glutamate?

Glutamine synthesis requires one mol of ATP



glutamate

glutamine

2nd way of NH_3 detoxication

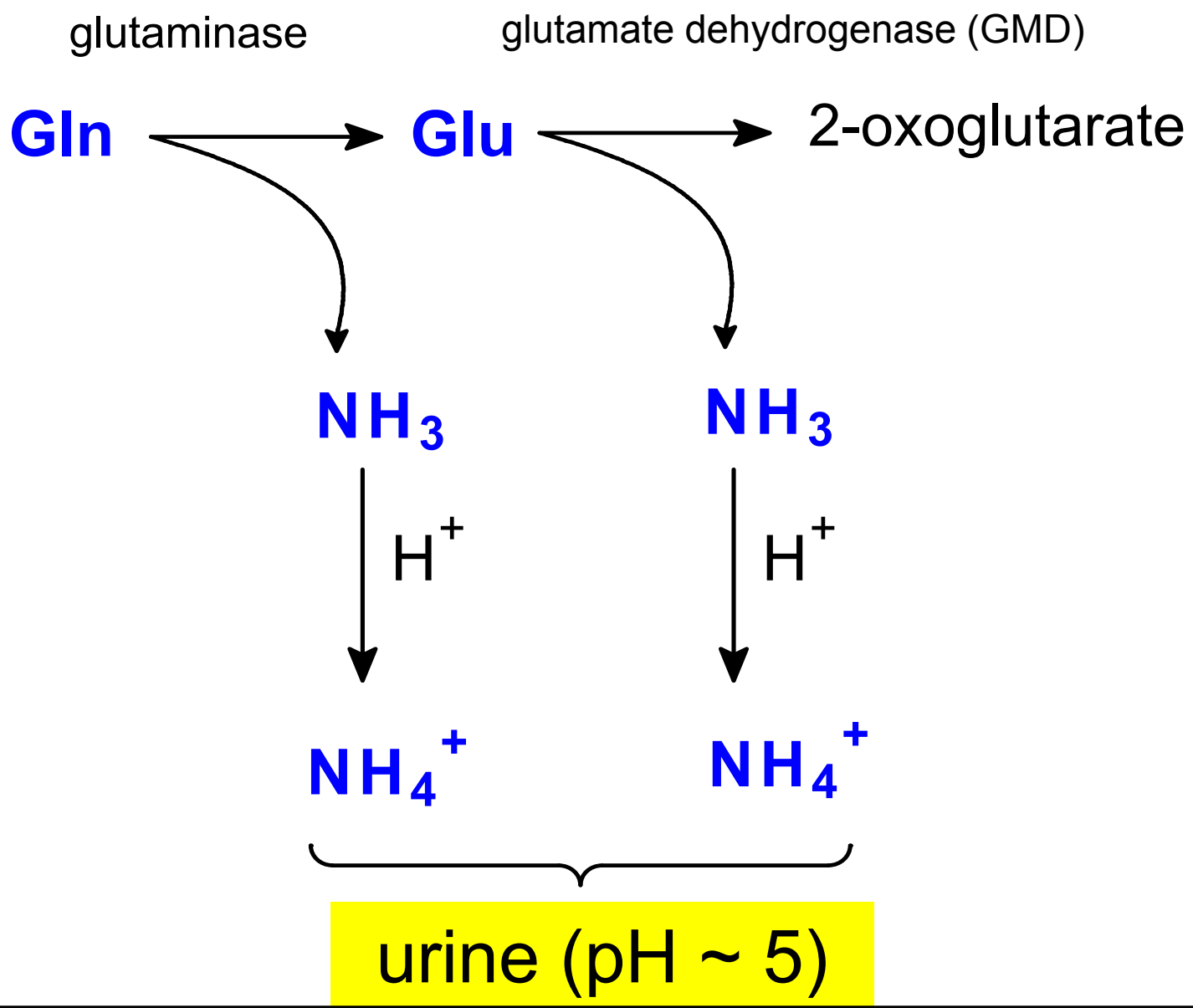
A. 19 – Gln in liver

- **in periportal hepatocytes**, Gln is the source of ammonia for urea synthesis
- **in perivenous hepatocytes**, Gln is made from glutamate (Glu + NH_3 → Gln) as the additional way of ammonia detoxication
- Gln is released from liver to blood - transported to enterocytes and kidney

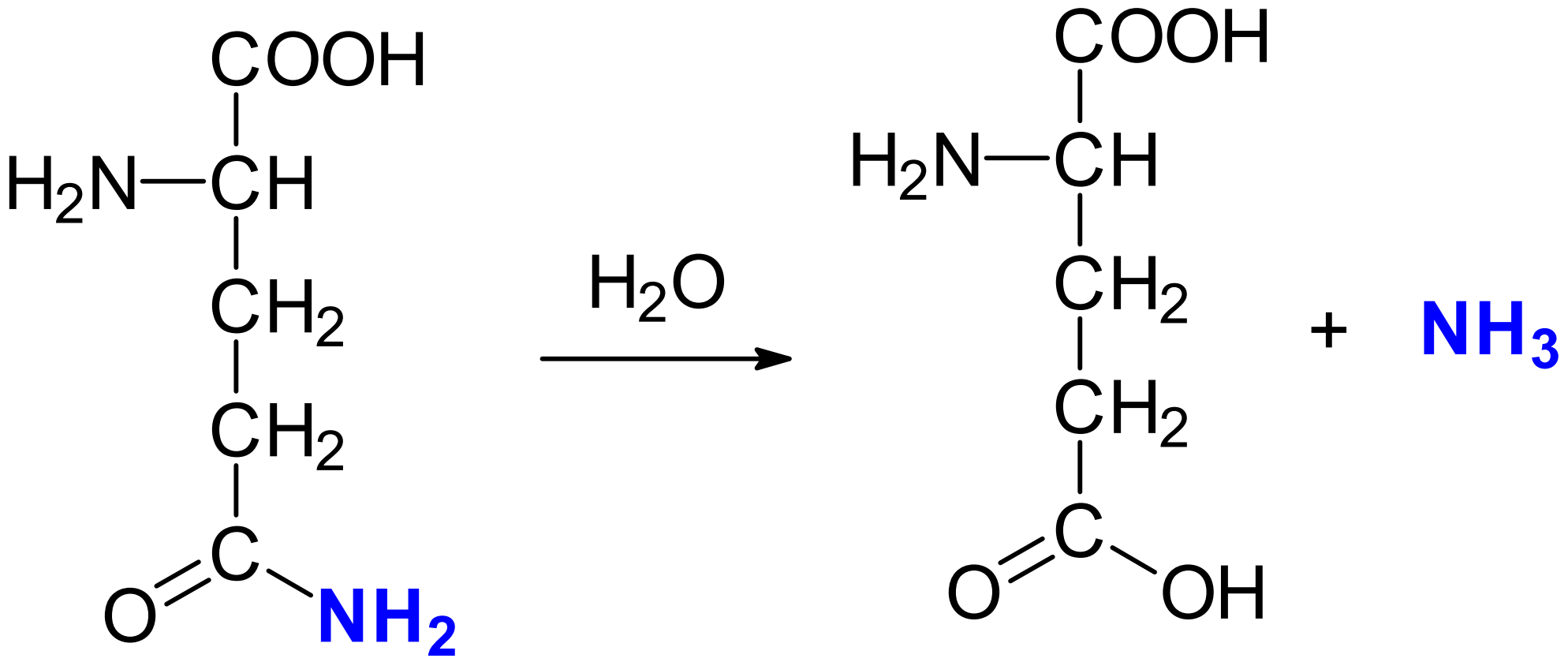
A. 19 – Gln in kidneys

- Gln is the source of energy for the kidneys, to a great extent especially in fasting and under metabolic acidosis
- Gln and Glu release ammonium ions which contribute to acidic pH of urine

The origin of ammonium in urine



Glutaminase catalyses the hydrolysis of amide group in glutamine



glutamine

glutamate

Multiple functions of glutamine

- Synthesis of proteins
- Metabolic fuel – enterocytes, lymphocytes, macrophages, fibroblasts, kidneys
- Source of nitrogen in synthesis – purine, pyrimidines, NAD^+, aminosugars
- Source of glutamate – GSH, GABA, ornithin, prolin,
- Source of ammonium ions in urine

Q. 20

A. 20 - AA in blood

Resorption phase

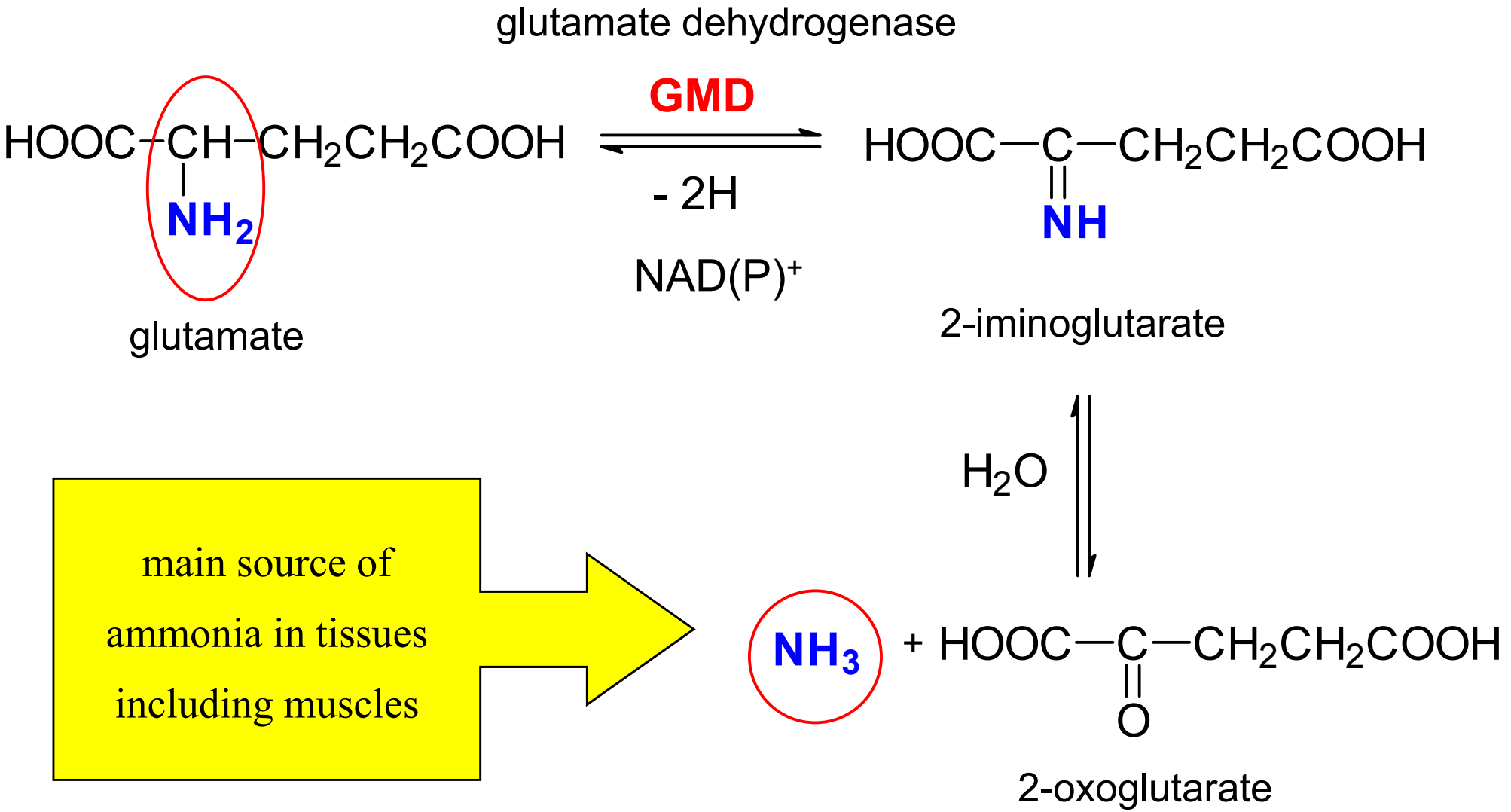
- predominate Val, Leu, Ile
- liver does not take them up from circulation (no specific aminotransferases in liver for Val, Leu, Ile)

Postresorption phase and fasting

- predominate Gln and Ala
- released from muscles (Gln + Ala) and liver (Gln)

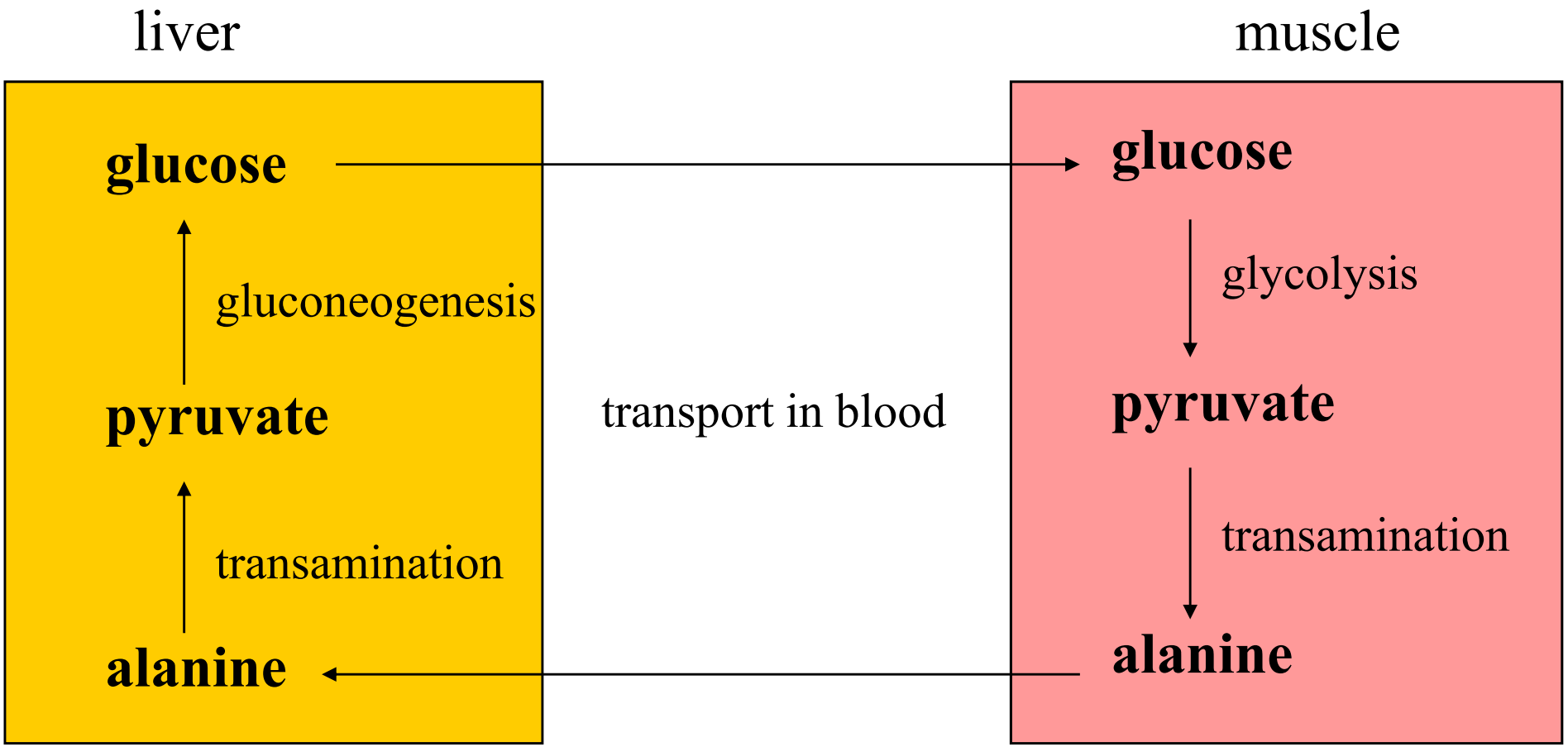
Q. 21

A. 21 - Dehydrogenation deamination of glutamate



Q. 22

A. 22 Glucose-alanine cycle



Q. 23

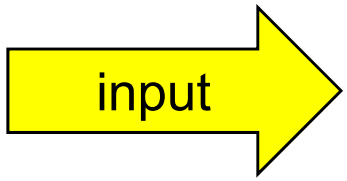
A. 23 Three ways of ammonia detoxication

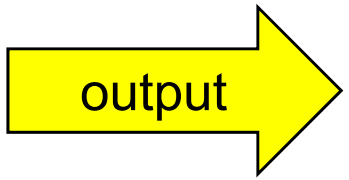
Feature	Urea	Glutamine (Gln)	Glutamate (Glu)
Relevance	★ ★ ★ ★ ★ ★	★ ★ ★ ★	★
Compound type	H_2CO_3 diamide	γ-amide of Glu	α-amino acid
Reaction(s)	urea cycle	Glu + NH_3	hydrog. amin. 2-OG
Enzyme	5 enzymes	Gln-synthase	GMD
Energy needs	3 ATP	1 ATP	1 NADH[a]
Organelle(s)	mitoch. + cytosol	cytosol	mitochondria
Organ(s)	only liver	liver, **brain**, other	(brain)

[a] Equivalent of 3 ATP (compare respiratory chain).

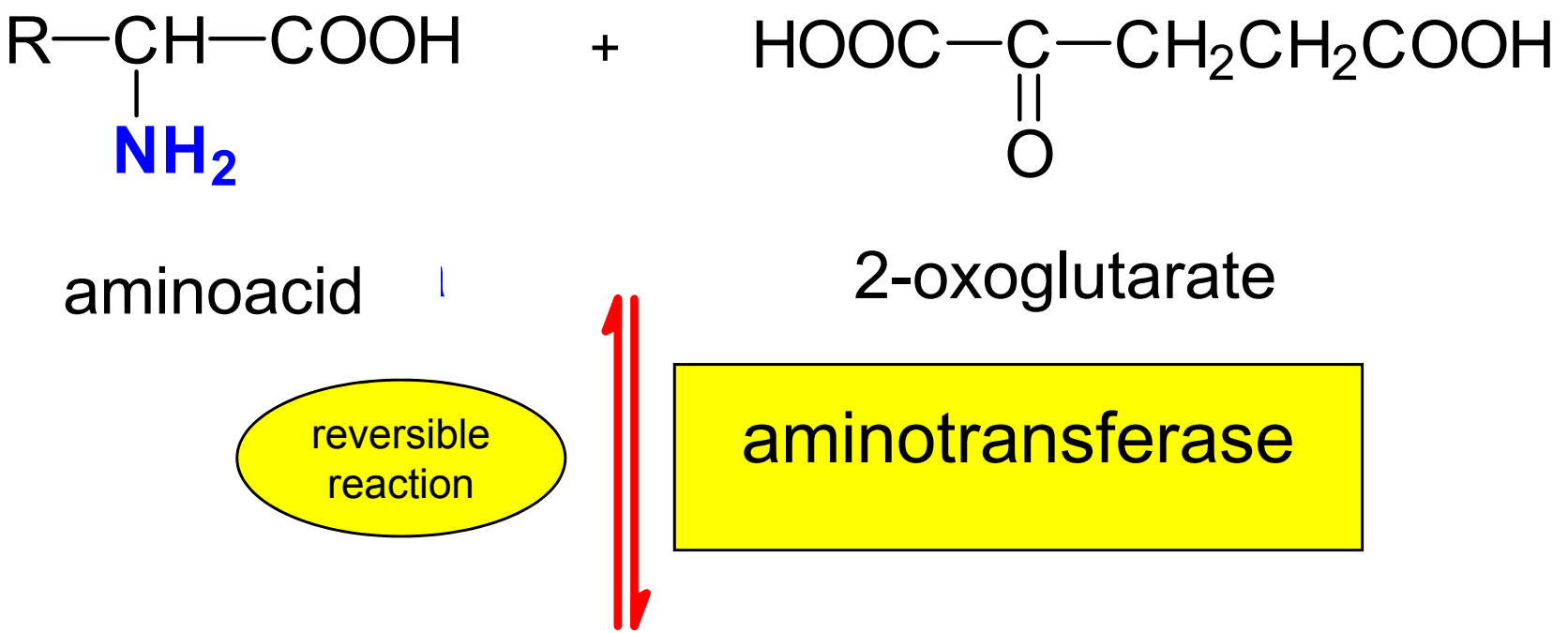
Q. 26

A. 26 Catabolic pathway of nitrogen (in blue colour)

- 

dietary **proteins** → **AA** (GIT)
- **transamination** of AA in cells → **glutamate**
- dehydrogenation deamination of glutamate → **NH_3**
- detoxication of ammonia → **urea** 

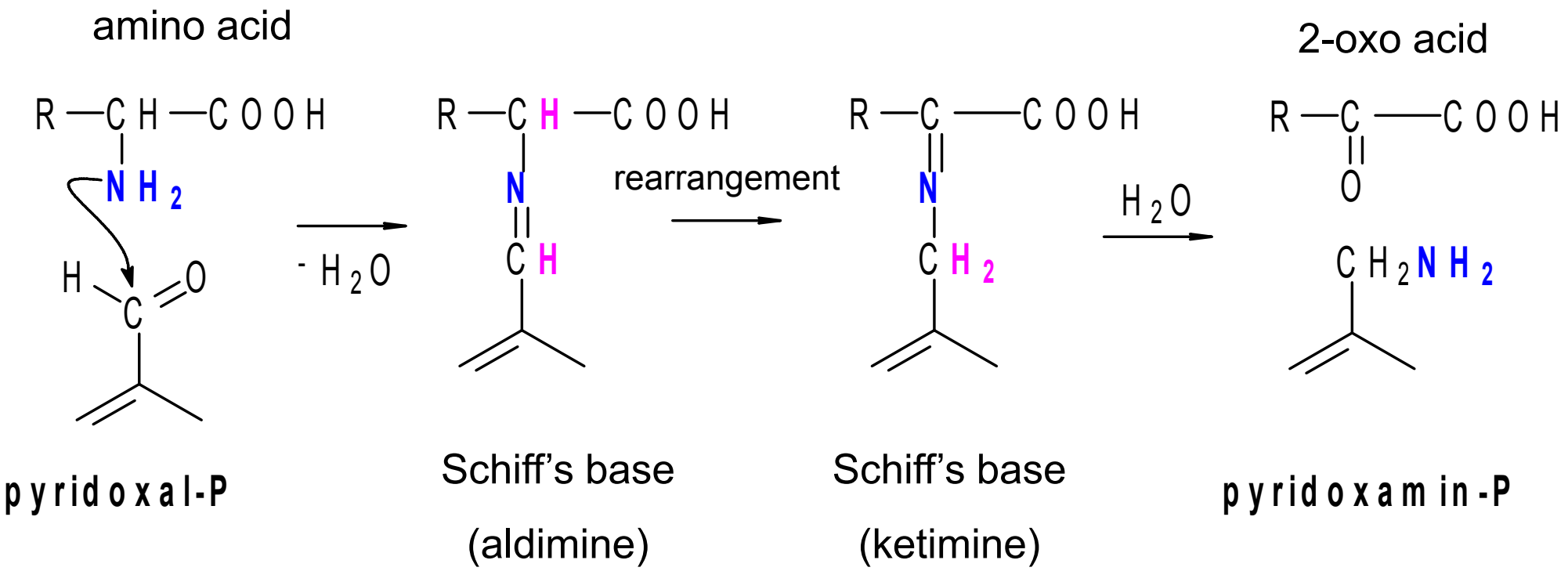
A. 26 General scheme of transamination



1. Phase of transamination

amino acid → oxo acid

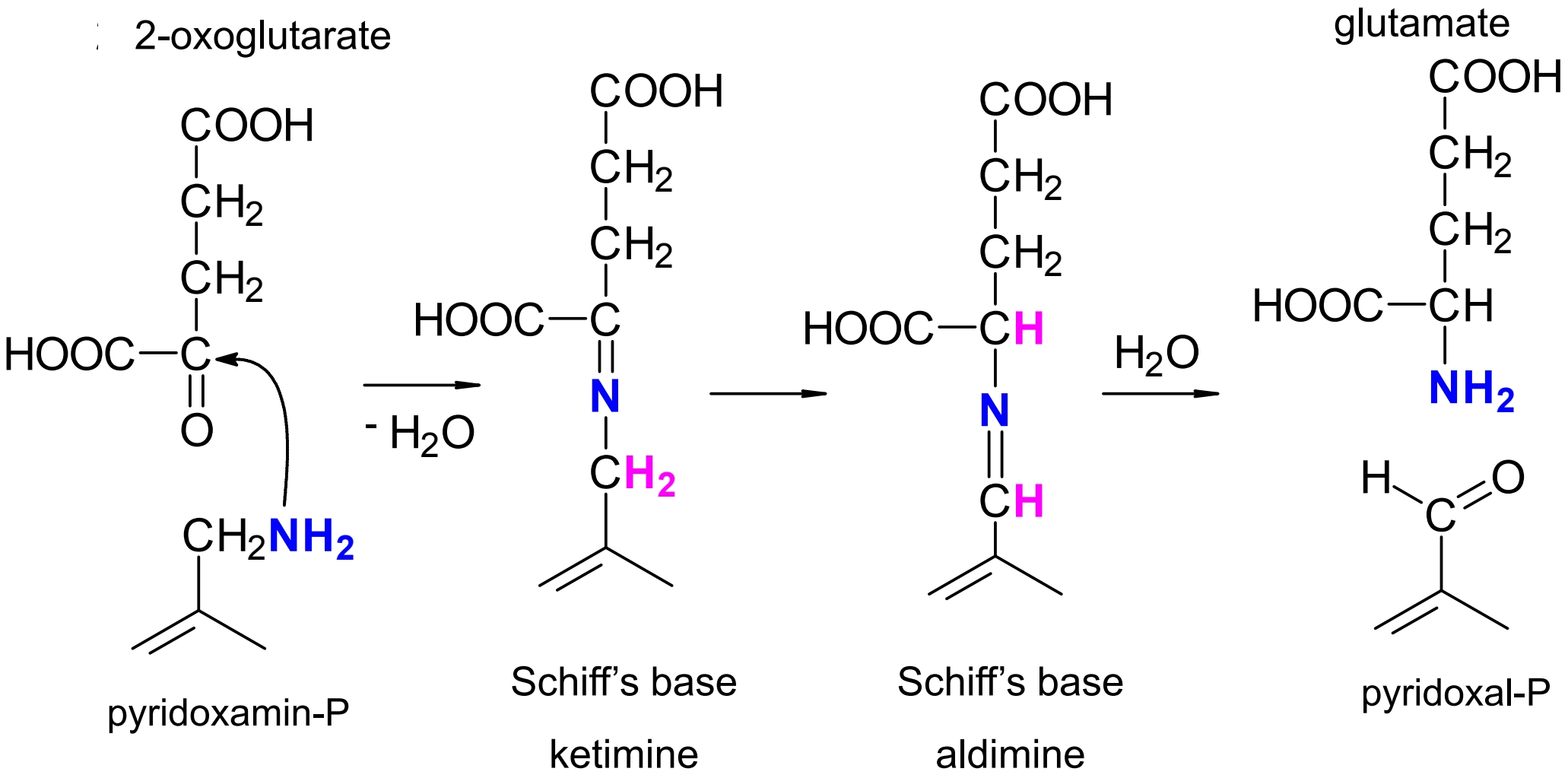
pyridoxal-P → pyridoxamine-P



2. Phase of transamination

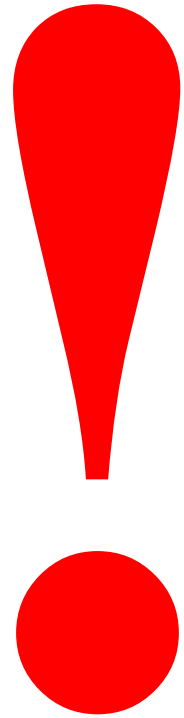
2-oxoglutarate → glutamate

pyridoxamine-P → pyridoxal-P



In transaminations, nitrogen of most AA is concentrated in glutamate

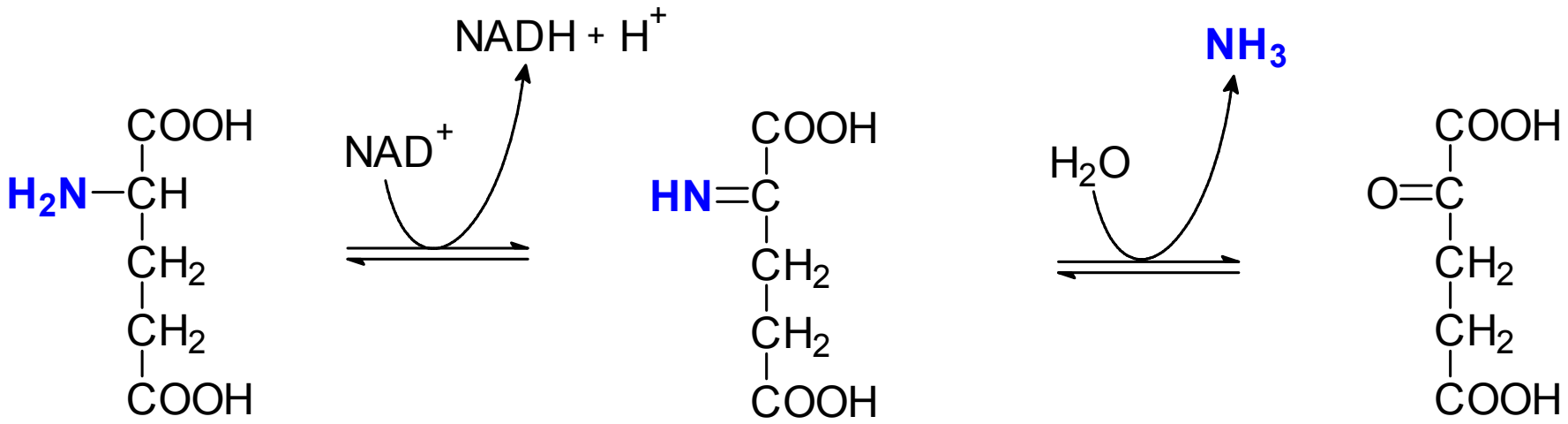
Glutamate then undergoes dehydrogenation deamination and releases **free ammonia NH_3**



GMD reaction is reversible

ammonia formation

dehydrogenation **deamination** **of glutamate**



hydrogenation **amination** **of 2-oxoglutarate**

ammonia detoxication

A. 27

A. 27

aspartate + 2-oxoglutarate ⇆ oxaloacetate + glutamate

- AST reaction is reversible
- provides aspartate for the urea synthesis

Q. 28

A. 28

ammonia ↑

glutamine ↑

Q. 29

$Hb\text{-}Val\text{-}NH_2 + NH_2\text{-}CO\text{-}NH_2 \rightarrow$

A. 29

$$Hb\text{-}Val\text{-}NH_2 + NH_2\text{-}CO\text{-}NH_2 \rightarrow Hb\text{-}Val\text{-}NH\text{-}CO\text{-}NH_2 + NH_3$$

Compound	Metabolic origin	Excretion by urine
Urea		330-600 mmol/day
Creatinine		5-18 mmol/day
NH_4^+		20-50 mmol/day
Uric acid		1-1.5 mmol/day
Free AA		4-14 mmol/d (α-amino N)

Compound	Metabolic origin	Excretion by urine
Urea	detoxication of NH_3 in liver	330-600 mmol/day
Creatinine	creatine catabolism (muscles)	5-18 mmol/day
NH_4^+	glutaminase and GMD reaction in kidney tubules	20-50 mmol/day
Uric acid	purine bases catabolism	1-1.5 mmol/day
Free AA	proteolysis in tissues	4-14 mmol/d (α-amino N)

Factors affecting nitrogen balance

Factor	ΔN
Growth, pregnancy	
Metabolic stress	
Starvation	
Incomplete food proteins	

Factors affecting nitrogen balance

Factor	ΔN
Growth, pregnancy	**positive**
Metabolic stress	**negative**
Starvation	**negative**
Incomplete food proteins	**negative**

Q. 30

A. 30

The loss of N = 4 g/day

Average content of N in proteins is 16 %.

Average content of proteins in muscles is 20 %.

---

100 g prot. ..... 16 g N

x g prot. .....4 g N

x = 400 / 16 = **25 g of proteins**

100 g muscles ..... 20 g proteins

x g muscles ..... 25 g proteins

x = 2500 / 20 = **125 g of muscles**