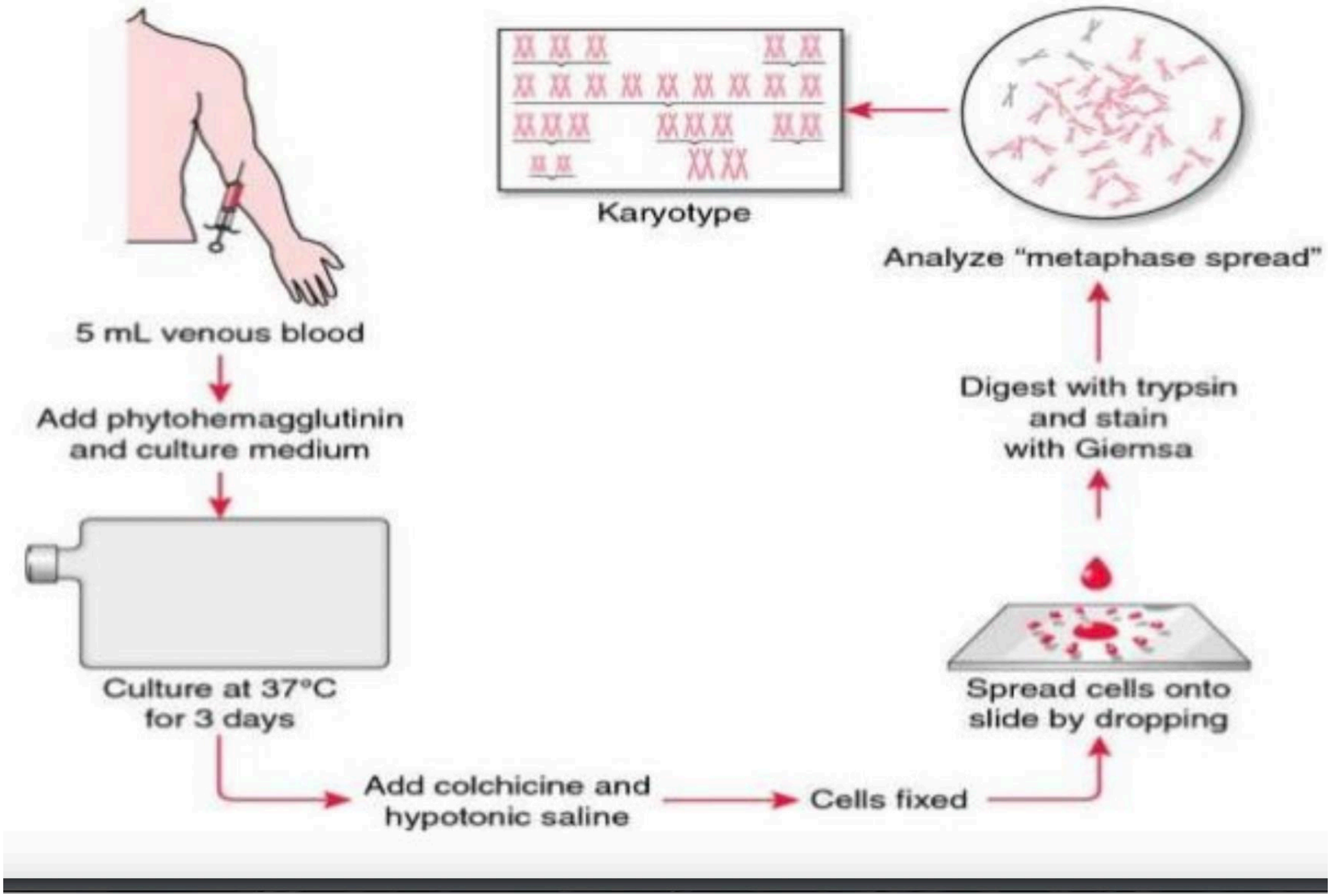
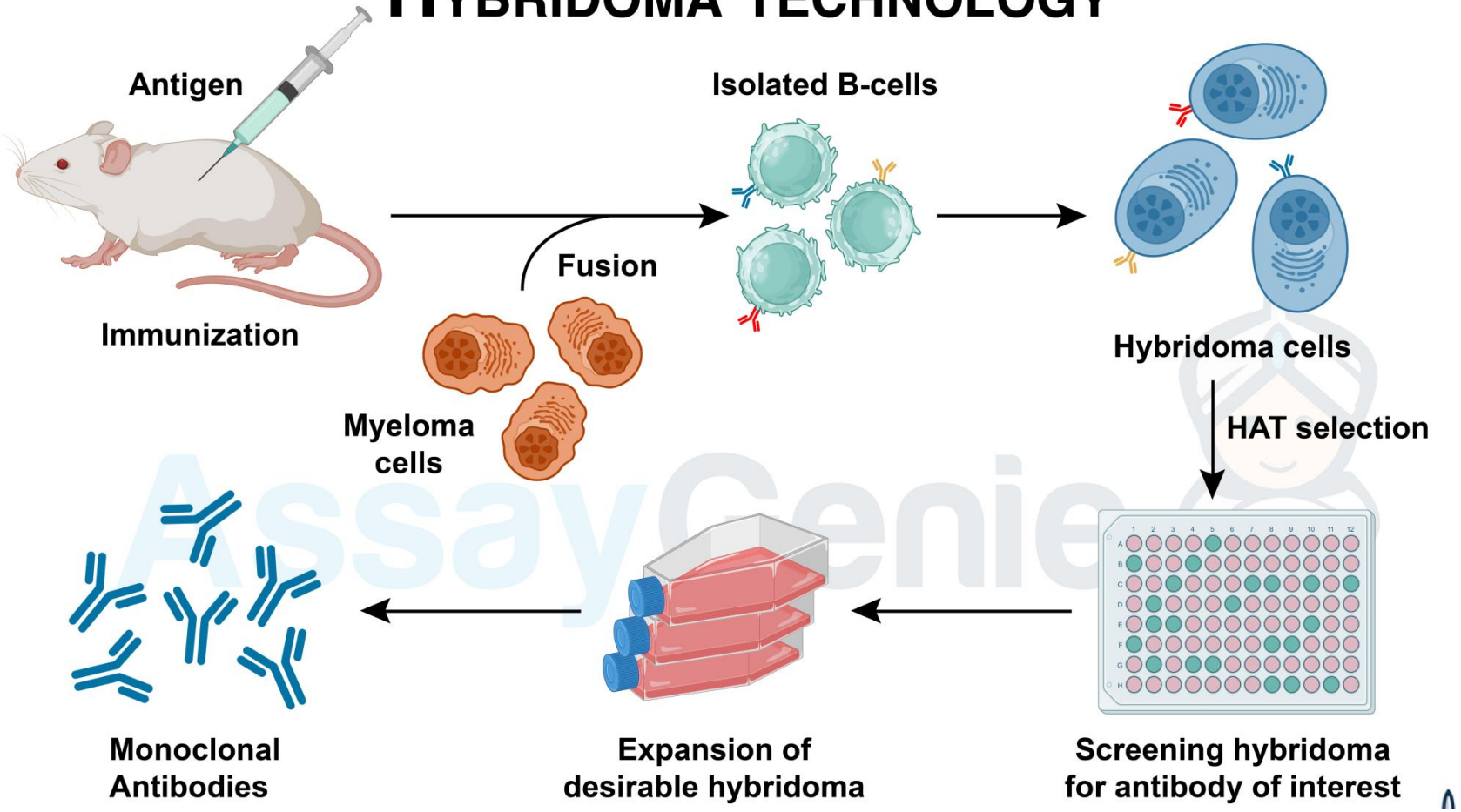
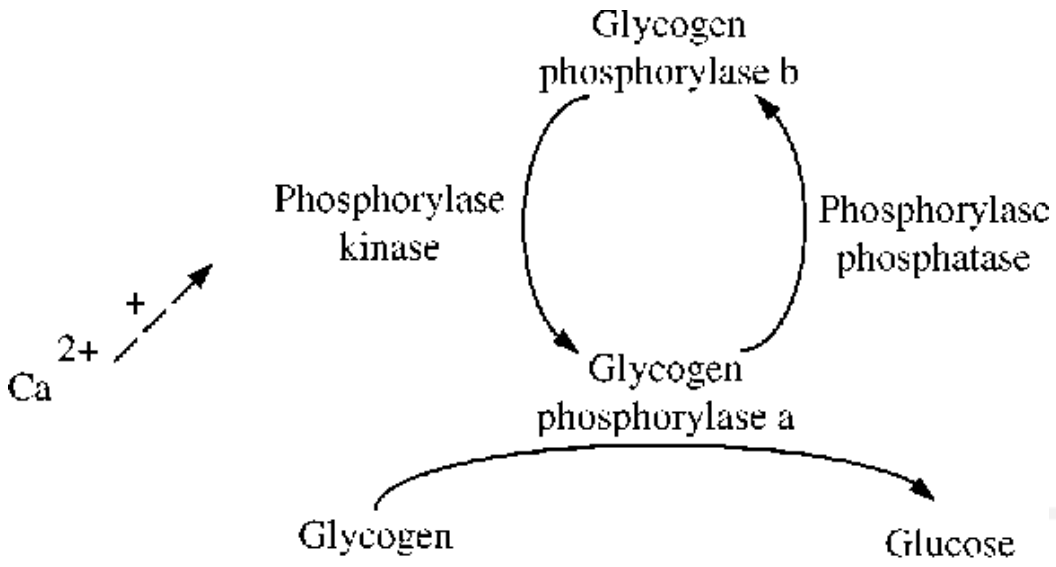
BIOCHEMISTRY BINGE REVISION

Medsynapse by Dr. Nikita

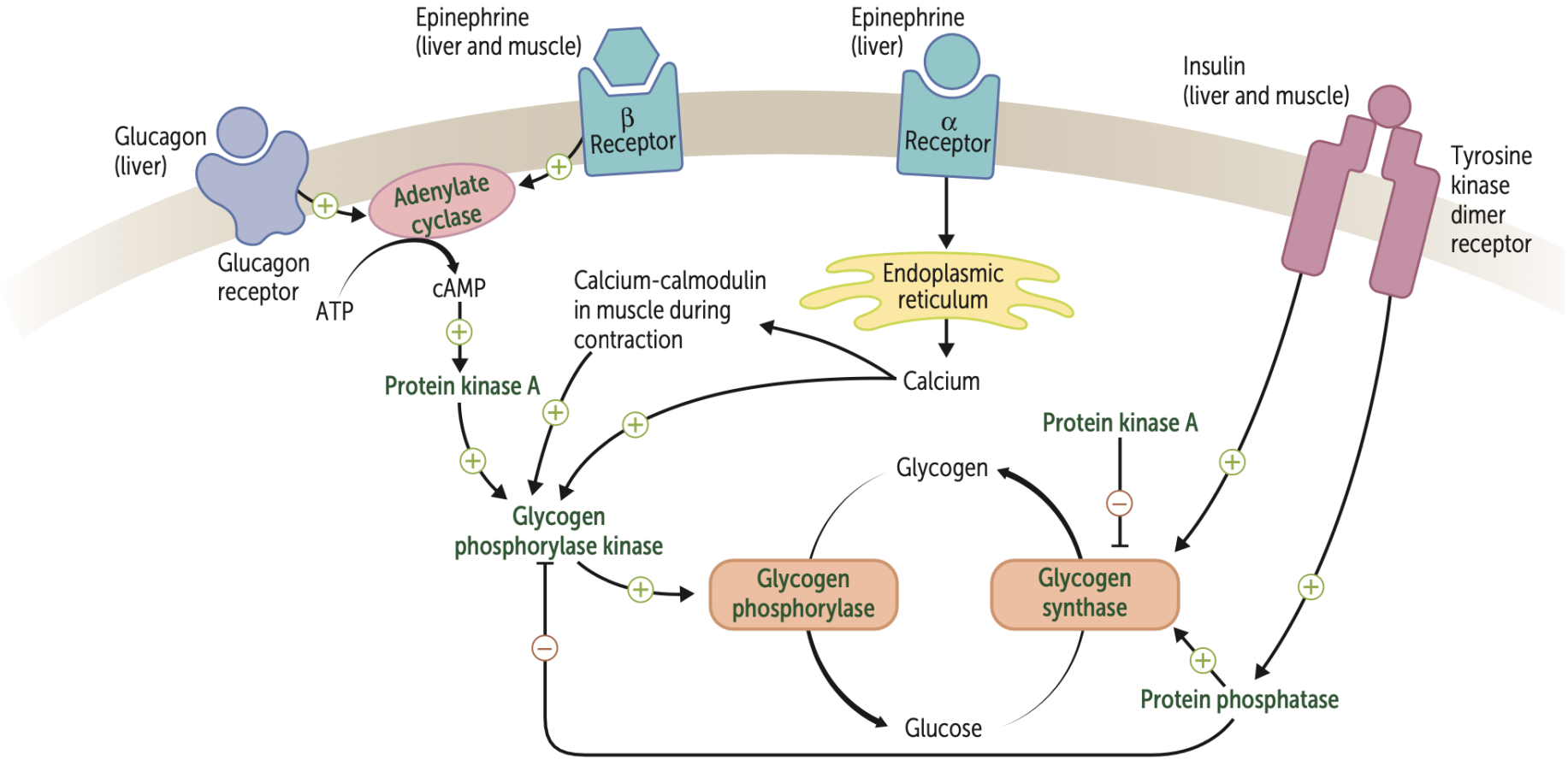


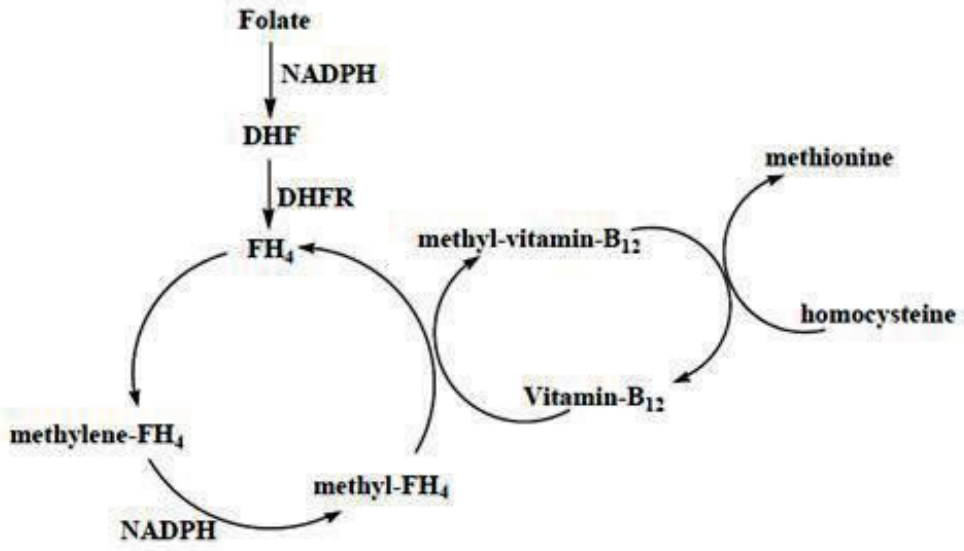
HYBRIDOMA TECHNOLOGY





Glycogen regulation by insulin and glucagon/epinephrine





Nucleoside = base + (deoxy)ribose (sugar).
Nucleotide = base + (deoxy)ribose + phosphate; linked by 3′-5′ phosphodiester bond.

5′ end of incoming nucleotide bears the triphosphate (energy source for the bond). α-Phosphate is target of 3′ hydroxyl attack.

Purines (A,G)—2 rings.
Pyrimidines (C,U,T)—1 ring.

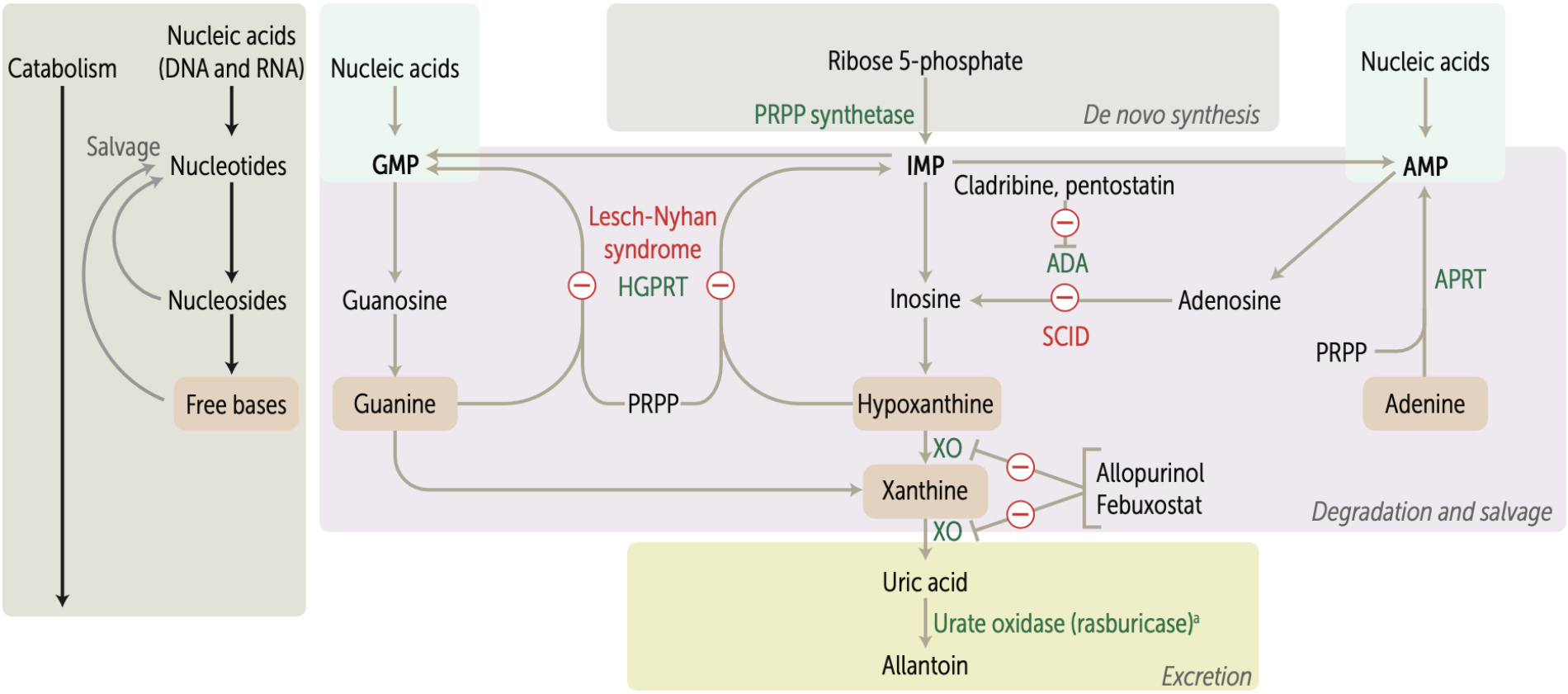
Pure As Gold.
CUT the pyramid.
Thymine has a methyl.

Deamination reactions:
- Cytosine → uracil
- Adenine → hypoxanthine
- Guanine → xanthine
- 5-methylcytosine → thymine

C-G bond (3 H bonds) stronger than A-T bond (2 H bonds). ↑ C-G content → ↑ melting temperature of DNA. "C-G bonds are like Crazy Glue."

Uracil found in RNA; thymine in DNA. Methylation of uracil makes thymine.

Amino acids necessary for purine synthesis (cats purr until they GAG):
- Glycine
- Aspartate
- Glutamine

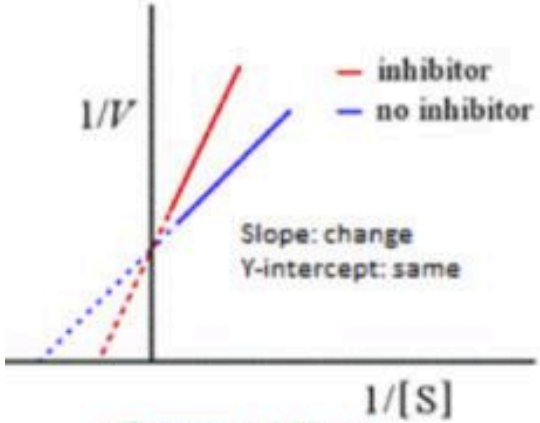


[a]Absent in humans.
ADA, adenosine deaminase; APRT, adenine phosphoribosyltransferase; HGPRT, hypoxanthine guanine phosphoribosyltransferase, XO, xanthine

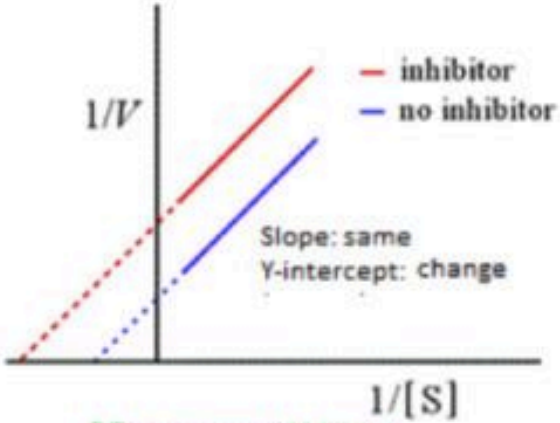
Regulation of gene expression

Promoter	Site where RNA polymerase II and multiple other transcription factors bind to DNA upstream from gene locus (AT-rich upstream sequence with TATA and CAAT boxes, which differ between eukaryotes and prokaryotes). Promoters increase ori activity.	Promoter mutation commonly results in dramatic ↓ in level of gene transcription.
Enhancer	DNA locus where regulatory proteins ("**activators**") bind, **increasing** expression of a gene on the same chromosome.	Enhancers and silencers may be located close to, far from, or even within (in an intron) the gene whose expression they regulate.
Silencer	DNA locus where regulatory proteins ("**repressors**") bind, **decreasing** expression of a gene on the same chromosome.	
Epigenetics	Changes made to gene expression (heritable mitotically/meiotically) without a change in underlying DNA sequence.	Primary mechanisms of epigenetic change include DNA methylation, histone modification, and noncoding RNA.

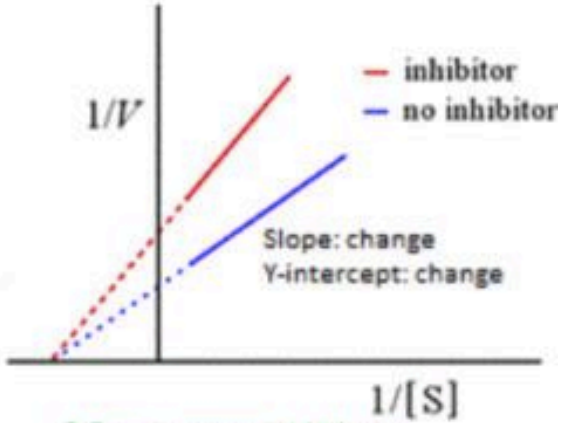
Lineweaver-Burk plots for enzyme inhibition



Competitive inhibition
K_M increased
V_{max} unaffected



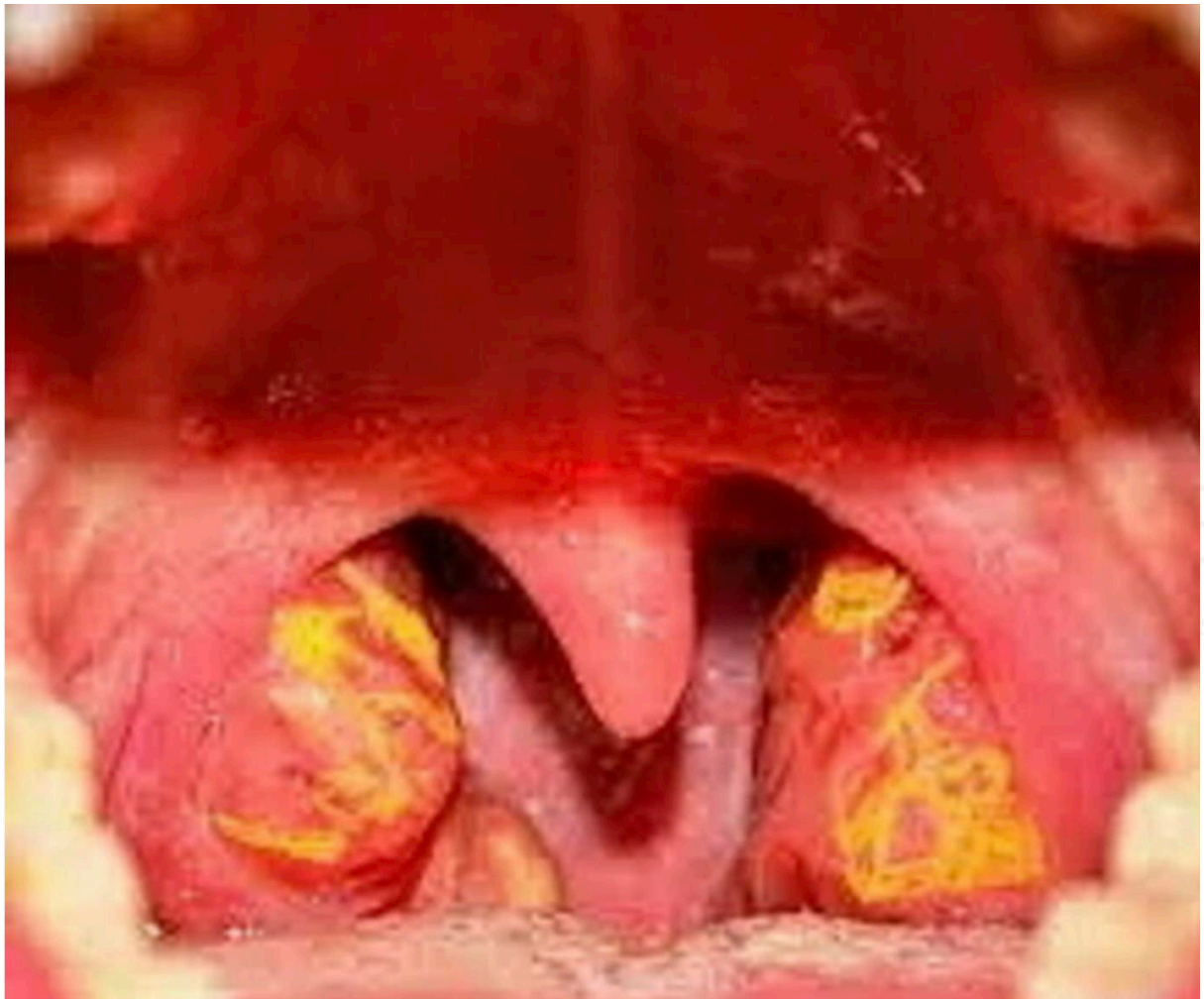
Uncompetitive inhibition
K_M reduced
V_{max} reduced



Noncompetitive
(Mixed) inhibition
K_M unaffected
V_{max} reduced

Rate-determining enzymes of metabolic processes

PROCESS	ENZYME	REGULATORS
Glycolysis	Phosphofructokinase-1 (PFK-1)	AMP ⊕, fructose-2,6-bisphosphate ⊕ ATP ⊖, citrate ⊖
Gluconeogenesis	Fructose-1,6-bisphosphatase 1	AMP ⊖, fructose-2,6-bisphosphate ⊖
TCA cycle	Isocitrate dehydrogenase	ADP ⊕ ATP ⊖, NADH ⊖
Glycogenesis	Glycogen synthase	Glucose-6-phosphate ⊕, insulin ⊕, cortisol ⊕ Epinephrine ⊖, glucagon ⊖
Glycogenolysis	Glycogen phosphorylase	Epinephrine ⊕, glucagon ⊕, AMP ⊕ Glucose-6-phosphate ⊖, insulin ⊖, ATP ⊖
HMP shunt	Glucose-6-phosphate dehydrogenase (G6PD)	$NADP^+$ ⊕ NADPH ⊖
De novo pyrimidine synthesis	Carbamoyl phosphate synthetase II	ATP ⊕, PRPP ⊕ UTP ⊖
De novo purine synthesis	Glutamine-phosphoribosylpyrophosphate (PRPP) amidotransferase	AMP ⊖, inosine monophosphate (IMP) ⊖, GMP ⊖
Urea cycle	Carbamoyl phosphate synthetase I	*N*-acetylglutamate ⊕
Fatty acid synthesis	Acetyl-CoA carboxylase (ACC)	Insulin ⊕, citrate ⊕ Glucagon ⊖, palmitoyl-CoA ⊖
Fatty acid oxidation	Carnitine acyltransferase I	Malonyl-CoA ⊖
Ketogenesis	HMG-CoA synthase (HOMG! I'm starving!)	
Cholesterol synthesis	HMG-CoA reductase	Insulin ⊕, thyroxine ⊕, estrogen ⊕ Glucagon ⊖, cholesterol ⊖



Peroxisome

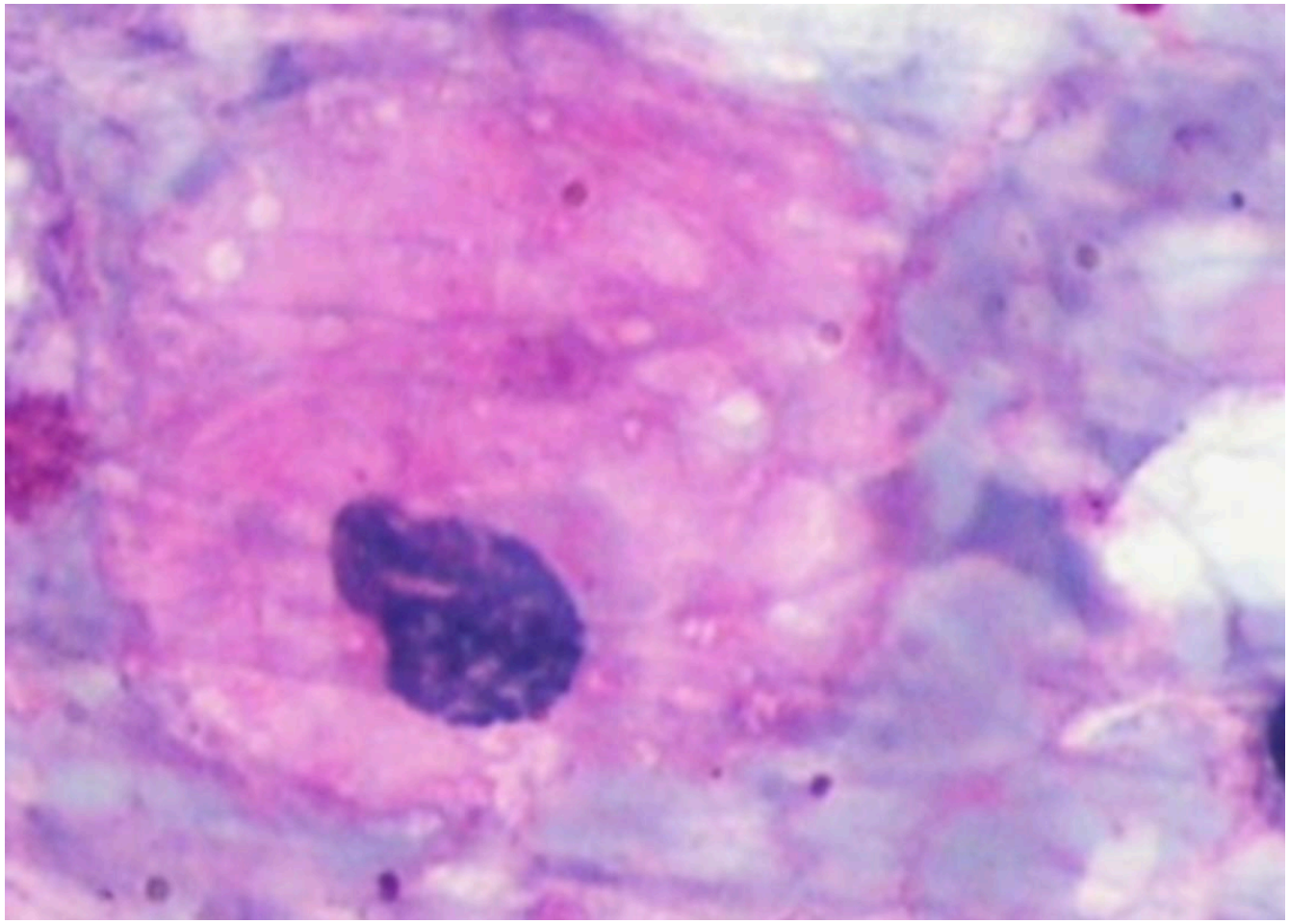
Membrane-enclosed organelle involved in:

- β-oxidation of very-long-chain fatty acids (VLCFA) (strictly peroxisomal process)
- α-oxidation of branched-chain fatty acids (strictly peroxisomal process)
- Catabolism of amino acids and ethanol
- Synthesis of bile acids and plasmalogens (important membrane phospholipid, especially in white matter of brain)

Zellweger syndrome—autosomal recessive disorder of peroxisome biogenesis due to mutated *PEX* genes. Hypotonia, seizures, jaundice, craniofacial dysmorphia, hepatomegaly, early death.

Refsum disease—autosomal recessive disorder of α-oxidation → buildup of phytanic acid due to inability to degrade it. Scaly skin, ataxia, cataracts/night blindness, shortening of 4th toe, epiphyseal dysplasia. Treatment: diet, plasmapheresis.

Adrenoleukodystrophy—X-linked recessive disorder of β-oxidation due to mutation in *ABCD1* gene → VLCFA buildup in **adrenal** glands, white (**leuko**) matter of brain, testes. Progressive disease that can lead to adrenal gland crisis, progressive loss of neurologic function, death.



★ Pompes dis ⟶ enzyme = lysosomal ∝ glucosidase

★ HMP shunt produces ribose, does not utilise ribose

* Specific dynamic allowance / thermic effect is max ī **PROTEINS**

* **COLLAGEN** :
  1. Most abundant a.a = Glycine at every 3rd posn.
  2. Triple helical structure.
  3. Hydroxylase → vit C
  4. Lysyl Oxidase → Cu. → Mcnke
     cross linking

* 2, 3 BPG binds to deoxyHb → globin chain of Hb.
  O_2 binds to heme

* Substrate is complementary to active sit of enzyme.

* A.a restricted in MSUD :

* Burnt sugar odour :

* Selenocysteine : gist on : UGA

  Enzymes :
  - Glutathione peroxidase
  - Thioredoxin reductase
  - Deiodinase

① Phospholipids :

- Mitochondria → Cardiolipin (Diphosphatidyl glycerol)
  Def → (Barth syndrome)
- Brain → cephalin (phosphatidylethanolamine)
- HMD → Dipalmitoyl lecithin
- atherosclerosis → (oxidized lecithin)

② Liver lacks ——— and ∴ cannot use ketone bodies.
↓
Ketogenesis =

① RNA synthesis : . Clone of · Coding strand (same)
· Reverse of template stand.

Template
eg. → 5' CATTCG 3'

RNA · 3' GUAAGC 5'

ie 5' CGAAUG 3'

② FISH ——— determine DNA sequence alteration

. ✓ RFLP, pyrosequencing

× Flow cytometry
↳ CD marker

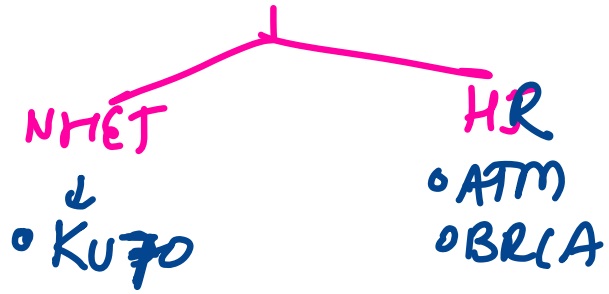
③ DNA repair :

① Xeroderma pig

② HNPCC (MLH1/MSH2)

③ SCID

④ Double stranded break repair



④ Hybridoma technology uses Salvage of purine synthesis (HGPRT dependent)

* To detect DNA - protein interaction : DNA footprinting

vs DNA fingerprinting : uses RFLP for paternity testing

* Humane genome project :
  - 1990 – 2003 : ~ 13 yrs
  - Total : 20500 genes
  - First generation sequencing like Sangers was used (Not next generation)
    L = array techniques.
  -

* Banding :
  - dicentric →
  - fluorescent →
  - Routine →
  - Telomere →

* . Nested PCR :
  - Multiplex PCR : for multiple genes at one time
  - Digital droplet PCR : for low freq. mutant allele
  - Sanger technique → sequencing → dideoxy NTP

* Deficiency of ——— leads to misincorporation of uracil into DNA.
  . one carbon carrier:

* Cystic fibrosis mutation: ΔF508, CFTR
  ↓ ↓
  phenylalanine Cl- channel

* Chimeric DNA is used for: organ transplant.

* RNA editing does not happen in 5' to 3' direction

- Sickle cell ~~fit~~ mutn:
- CRISPR – cas9 : NHEJ
- mRNA to ribosome binding : by guanyl cap
- Palindrome : AAGCTT
- Klenow fragment lacks : $5^{1}-3^{1}$ exonuclease
- Rothera test for ketones

* Phenylbutyrate is used in urea cycle disorders ∵ it scavenge nitrogen.
(phenyl butyl glutamine)

* Not seen in low insulin – glucagon:
  a) glycogen bkdown
  b) ketogenesis
  c) glycogen storage
  d) gluconeogenesis

① Xanthurenic aciduria = def of vit ——

② megaloblastic anemia ↓ ⓝ MMA ↑

* ③ vit E < def / toxicity

④ Raw egg → enzyme def → Pyruvate carboxylase

⑤ RBC transketolase → vit B_1

* Cyt. P450 utilises NADPH during phase 1
  - reach of xenobiotics detoxifies
  - (Hydroxylam)
  - ↓
  - oxidn reductn hydrolysis

* Increased H+ ions in intermembrane space of mitochondria due to → ↓ oxidative phosphorylation (by oligomycin)

* fructosamine → glycated albumin

- alternative to HbA1c (~12 wks control)
- 2 ~ 3 wks control