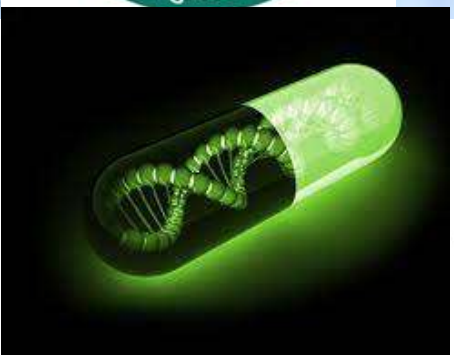
Department of Biochemistry

Newsletter

All India Institute of Medical Sciences,Rajkot



CLINIAL BIOCHEMISTRY & MOLECULAR BIOLOGY BULLETIN

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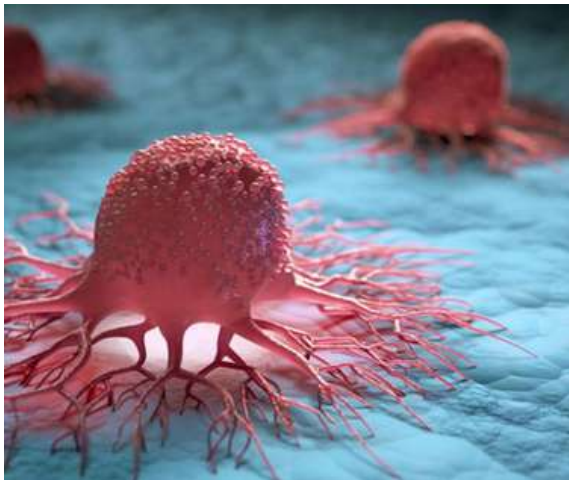
This issue is dedicated to

Role of AGE-RAGE in Cancer Progression

AGE-RAGE interaction elicits oxidative stress generation that evokes proliferative, angiogenic, and inflammatory reactions, thereby being involved in the development and progression of various types of cancers.

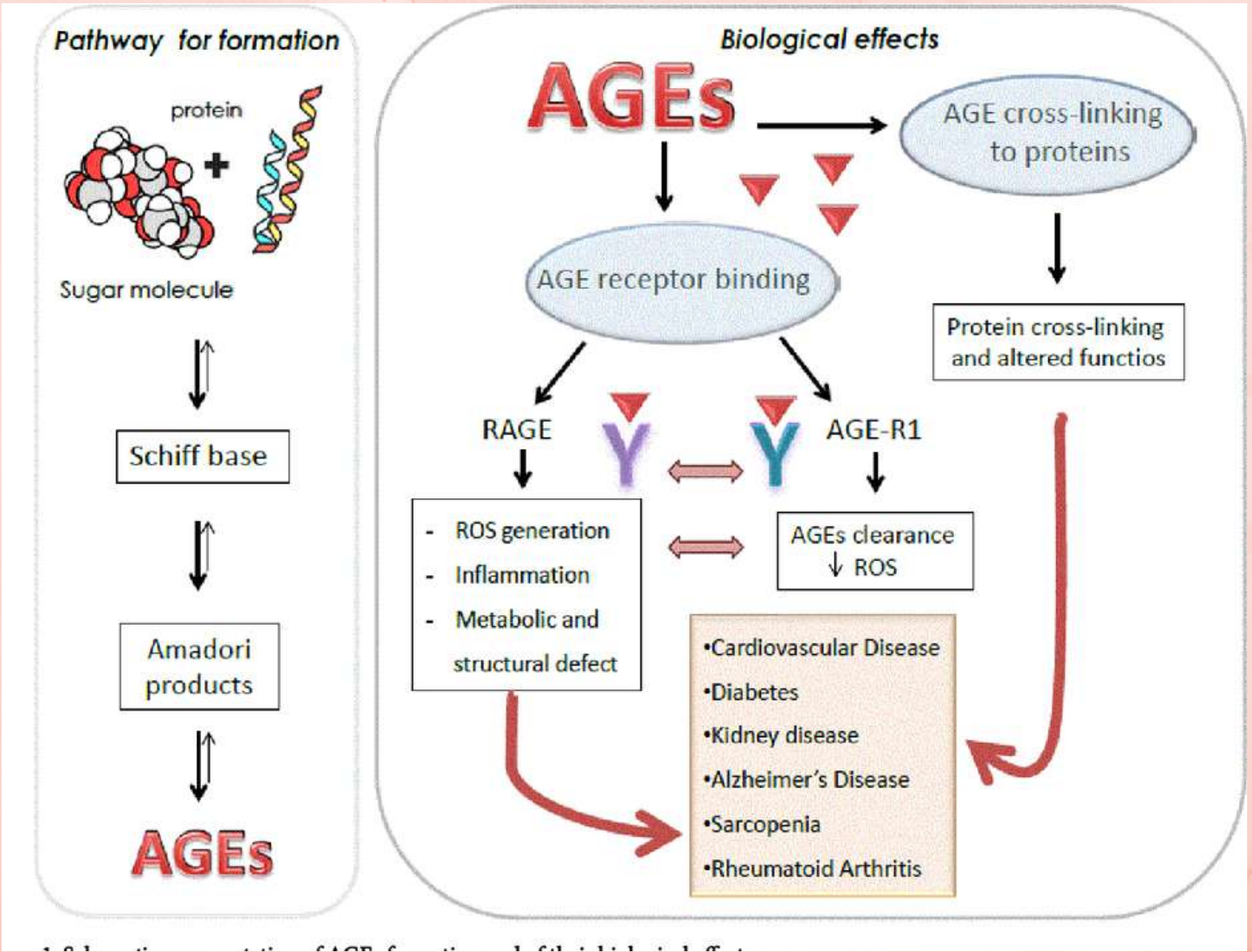


What Is AGE?

The non-enzymatic reaction of reducing sugars with the amino groups of proteins, lipids, and nucleic acids initiates a complex series of rearrangements and dehydration, producing a class of irreversibly cross-linked heterogeneous fluorescent moieties, named advanced glycation end products (AGEs).

AGEs, when accumulate in tissues, increase inflammation in the body which has an established association with carcinogenesis. The focus has now increased on factors promoting AGE formation, its reduction strategies, occurrence, & relevance of AGEs in cancer tissues along with the role of AGE-interaction with the Receptor for Advanced Glycation End-products (RAGE) in cancer initiation and progression.

In vivo glycation of proteins is physiological & is a type of post-translational modification occurring gradually but continuously throughout the life span. Under hyperglycemic conditions, such as that found in diabetes, AGE accumulation is accelerated.



Since it is a known fact that chronic inflammation, oxidative stress, and cancer are intrinsically linked, a likely contribution of AGEs to malignant cell transformation and the development and progression of cancer cannot be ruled out.

How are AGEs formed?

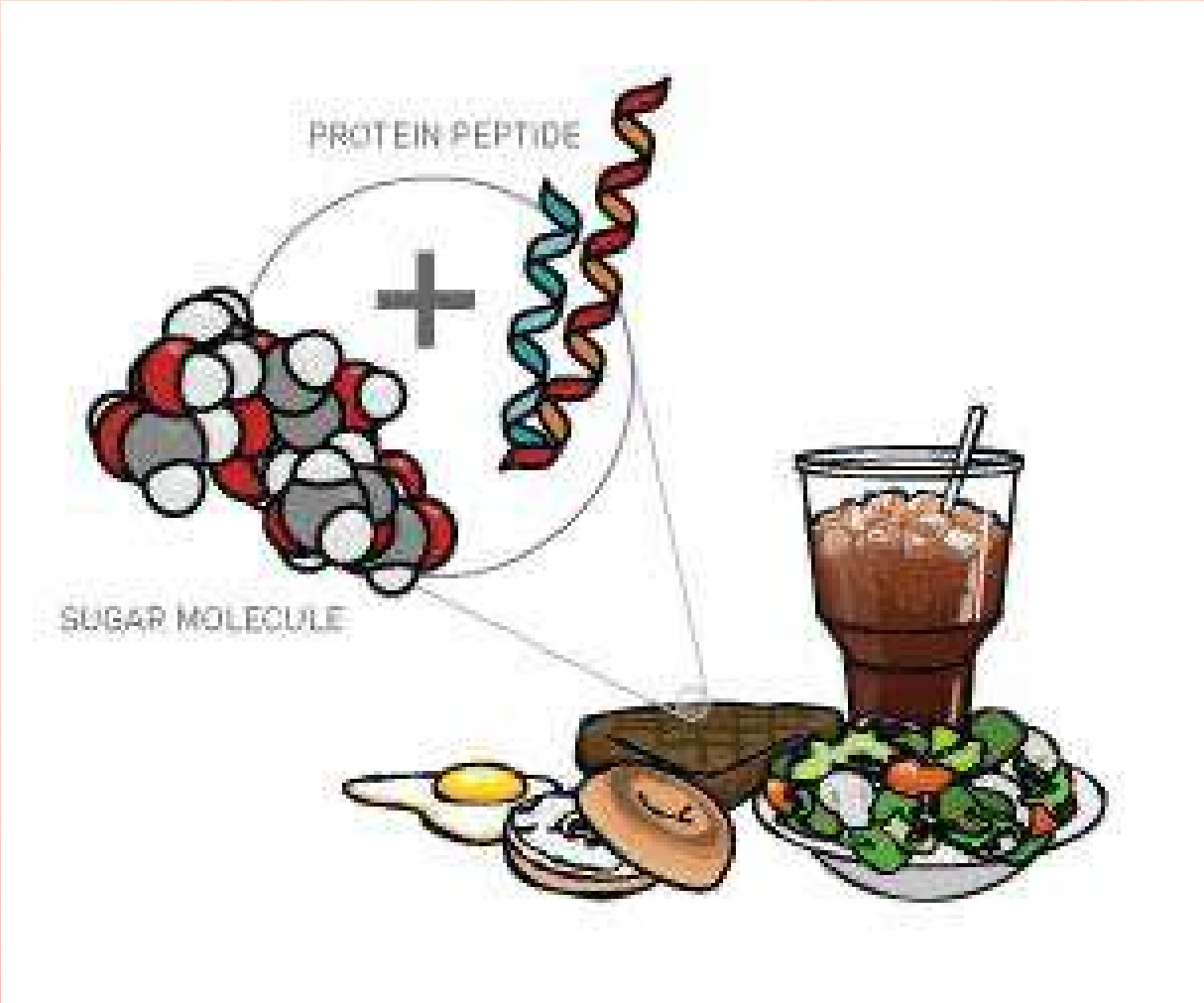
AGEs are formed endogenously via complex heterogeneous chemical reactions. The underlying mechanism is the Maillard reaction, occurring at different rates depending on temperature, pH value, & the respective sugar reactant.

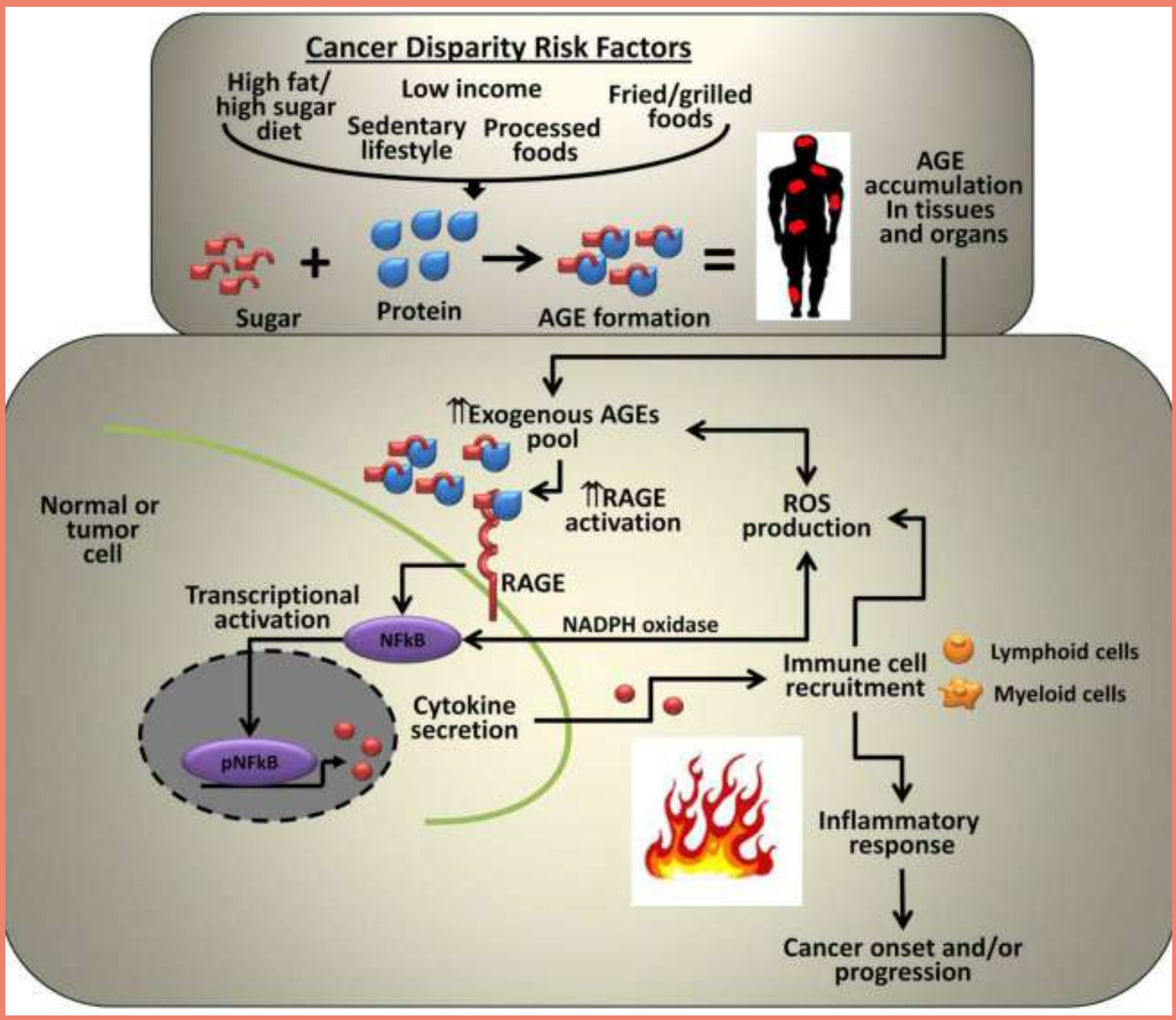
Most commonly, the formation of highly reactive *α-dicarbonyls (e.g. 3-deoxyglucosone (3-DG), methylglyoxal (MGO), & glyoxal (GO))* initiates the formation of AGEs

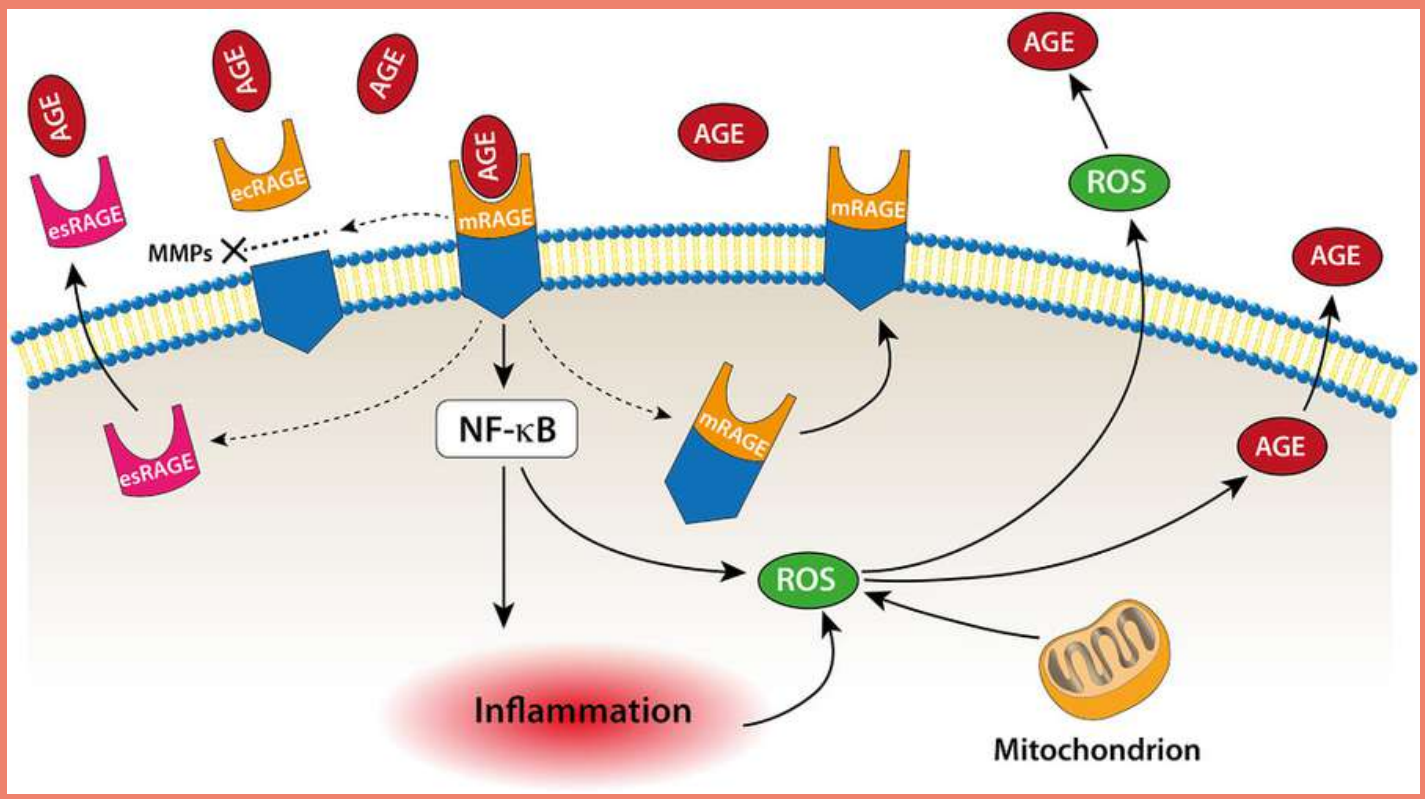
Lysine and arginine residues are the most susceptible glycation sites. In addition, cysteine, tryptophan, & histidine residues are favorable glycation sites too.

Also, lipid oxidation might also be initiated by the glycosylation of phospholipids, indicating that AGE formation can trigger the formation of ALEs (Advanced Lipoxidation End Products)

In combination, these pathways might increase the severity of inflammatory diseases, and the initiation and progression of malignant tumors.




Cancer Disparity Risk Factors
High fat/ high sugar diet
Low income
Sedentary lifestyle
Processed foods
Fried/grilled foods
Sugar
Protein
AGE formation
AGE accumulation In tissues and organs
Exogenous AGEs pool
RAGE activation
ROS production
Normal or tumor cell
RAGE
Transcriptional activation
NFkB
NADPH oxidase
Immune cell recruitment
Lymphoid cells
Myeloid cells
Cytokine secretion
pNFkB
Inflammatory response
Cancer onset and/or progression


AGE
esRAGE
ecRAGE
mRAGE
MMPs
NF-κB
ROS
Inflammation
Mitochondrion

Inflammation, RAGE & Cancer

- **RAGE is a Multiligand Single Transmembrane receptor and a member of the Ig superfamily of cell surface molecules. It also binds several other molecules such as β-amyloid peptides and β-sheet fibrils, prions, etc.**
- **By binding to the receptor these molecules stimulate signal transduction via a multitude of pathways like Ras-extracellular signal-regulating kinase 1/2, CDC42/Rac, p38 mitogen-activating protein kinase, NADPH-oxidase, and JAK1/2.**
- **Downstream signaling activates members of the STAT (signal transducers and activators of transcription) family, AP-1 (activator protein-1), & NFκB, a key target of RAGE signaling.**
- **Also, the AGE-RAGE interaction activates NADPH oxidase, causing increased intracellular oxidative stress.**
- **The occurrence of AGEs in human tumors was first shown in squamous cell carcinomas of the larynx, adenocarcinomas of the breast, adenocarcinomas of the colon, and leiomyosarcomas**

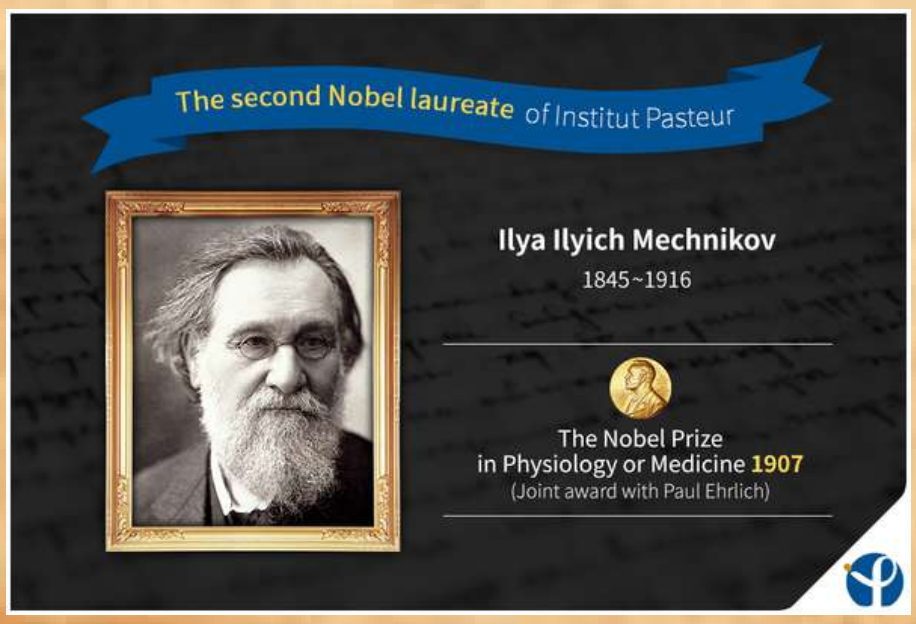
Strategies for AGE Reduction

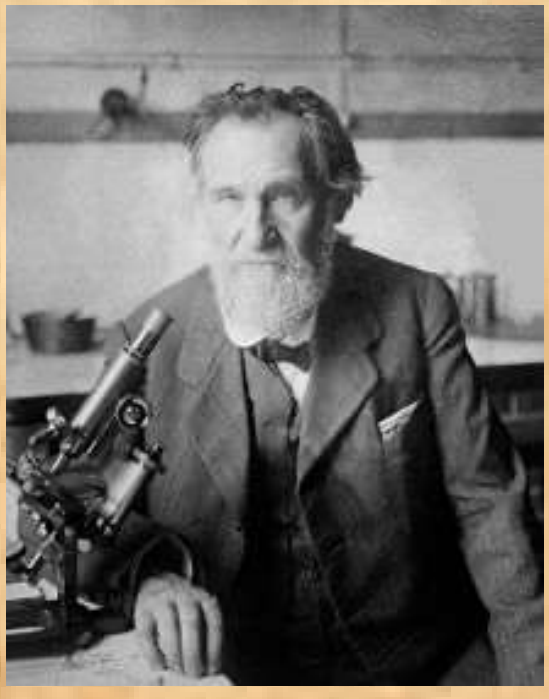
- **Strategies to reduce AGEs/ALEs formation in vivo include metal chelators and antioxidants.**
- **A diet rich in antioxidants (e.g. polyphenols and α-tocopherol as found in the Mediterranean diet) was shown to positively impact oxidative stress and inflammatory diseases by decreasing AGE formation in food and in vivo**
- **In addition, several metal-chelators such as Diethylenetria-minepentaacidic Acid (DETAPAC), Desferrioxamine (DFO), and triethylenetetramine (TETA) might inhibit the formation of AGES in Vitro and in vivo**
- **Other strategies to reduce AGE accumulation and RAGE activation in vivo comprise cross-link breakers (e.g. Alagebrium (ALT-711)), AGE adsorbents (AST-120 (Kremezin) and galectin-3), anti-hypertensive drugs, statins and bisphosphonates, respectively.**

Tid-Bits From History

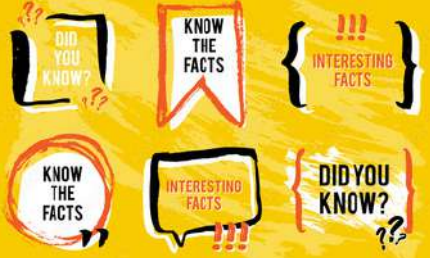
Father of Innate Immunity & Gerontology

Ilya Ilyich Mechnikov is honored as the "Father of Innate Immunity" & the "Father of Gerontology"
He was a Russian zoologist & was jointly awarded the 1908 Nobel Prize in Physiology or Medicine with Paul Ehrlich. He was the first to discover phagocytosis & phagocyte, specifically macrophages, in 1882.
He also developed one of the earliest concepts in aging and advocated the use of Lactobacillus for healthy and long life. This became the concept of probiotics in medicine. Mechnikov is also credited with coining the term Gerontology in 1903

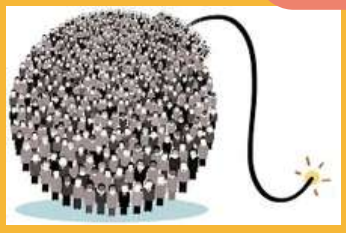




FUNFACT



Synthesis of Ammonia in the Lab is responsible for Population Explosion!!!



Did you know that the discovery of a way to make ammonia was the single most important reason for the world's population explosion from 1.6 billion in 1900 to 7 billion today?!

Nitrogen plays a crucial role in the Biochemistry of every living thing. It is also the most common gas in our atmosphere. But plants and animals can't extract it from the air. Consequently, a major limiting factor in agriculture has been the availability of nitrogen.

In 1910, German chemists Fritz Haber and Carl Bosch combined atmospheric nitrogen and hydrogen into ammonia. This in turn was fit to be used as crop fertilizer, eventually filtering up the food chain to us.

Today about 80% of the nitrogen in our bodies comes from the Haber-Bosch process, making this single chemical reaction probably the most important factor in the population explosion

Laboratory Touch-up

GLYOXALASE SYSTEM

The glyoxalase system is a set of enzymes that has a critical role in the prevention of glycation reactions mediated by methylglyoxal, glyoxal, and other alpha-oxoaldehydes.

- The glyoxalase system catalyzes the conversion of reactive, acyclic alpha-oxoaldehydes into the corresponding alpha-hydroxy acids. The mechanism is accomplished by the sequential action of two thiol-dependent enzymes:
  1. GLYOXALASE I (GLO-1)
  2. GLYOXALASE II

GLYOXALASE I (GLO-1), a lactoylglutathione lyase also known as methylglyoxalase, aldoketomutase, ketone-aldehyde mutase, and (R)-S-lactoylglutathione methylglyoxal-lyase.

Glyoxalase I is present in the cytosol of cells. It catalyzes the isomerization of the hemithioacetal, formed spontaneously from alpha-oxoaldehyde and GSH, to S -2-hydroxyacylglutathione derivatives [RCOCH(OH)-SG-->RCH(OH)CO-SG], and in so doing decreases the steady-state concentrations of physiological alpha-oxoaldehydes and associated glycation reactions.

- Physiological substrates of GLO0-1 are methylglyoxal, glyoxal, and other acyclic alpha-oxoaldehydes.
- Human GLO-1 is a dimeric Zn(2+) metalloenzyme of molecular mass 42 kDa.

The primary physiological function of GLO-1 is the detoxification of methylglyoxal, a reactive 2-oxoaldehyde that is cytostatic at low concentrations and cytotoxic at millimolar concentrations.

- GLO-1 is a target for the development of pharmaceuticals against human cancer.

Simple, direct, and automation-ready procedures for measuring GLO-1 activity in biological samples are highly desirable in research and drug discovery.

CALCULATION to Measure GLO-1 Activity:

For pure samples glyoxalase activity is calculated as:

$$\text{GLO-1} = \frac{OD_{10} - OD_0}{\varepsilon \times l} \times \frac{V_T}{t} \times \frac{1}{V_S} = 350 \times (OD_{10} - OD_0)\ (\text{U/L})$$

where OD(10) and OD(0) are the optical density values of the sample taken at 10 min and 0 min respectively. VT is the total reaction volume (0.2 mL), VS is the sample volume (40 µL), ε is the S-lactoylglutathione extinction coefficient (3.37 mM-1cm-1), l is the path length (0.425 cm for 0.2 mL in provided plate) and t is the reaction time (10 min).

For proteinous samples glyoxalase activity is calculated as:

$$\text{GLO-1} = \frac{OD_{10} - OD_0}{\varepsilon \times l} \times \frac{V_T}{t} \times \frac{1}{V_S} = 350 \times (OD_{10} - OD_0)\ (\text{U/L})$$

where OD(SAMPLE) and OD(BLANK) are the optical density values of the sample and sample blank respectively. t is the reaction time (20 min), 1.35 is the dilution factor for the deproteination step and n is the dilution factor if a sample dilution is required.

Unit definition: 1 unit of Glyoxalase-1 forms 1 µmole of S-lactoylglutathione from methylglyoxal and reduced glutathione per minute at pH 6.6 and 25°C.

Quote of the day

UPCOMING EVENT!