Carlton
Schwerdt
Chemistry 245
E.L. TATUM
Jan-Mar 1941

Comparative Biochemistry - Outline

I - Methods of obtaining energy
- evolutionary development
- mechanisms & reactions involved
    - respiratory mediators
    - similarity of mechanisms in various scales of life
    - nucleotides, coenzymes, enzymes, C_4 dibasic acids etc.
    - analogies of amino acid & carbohydrate catabolism.

II Utilization of energy for life.
- growth
- restoration of cell material, hence syntheses.

III Syntheses by cell
- carbohydrates, amino acids, proteins etc.
- inability to synthesize certain things (vitamins etc.) This represents a progressive (evolutionary) loss of power to synthesize by the organism.
- Vitamins probably function as catalysts

- ability to synthesize amino acids.
  - nature of essential + non-essential amino acids (that is, structural)

IV Enzymes

- enzymes control:-
  - structural characteristics of organisms
  - reaction " " "
- enzymes may be classed as:-
  - adaptive
  - constitutive
- production of enzymes is controlled genetically

V Some aspects of gene action.

- similarity to viruses + phage
  - size, composition, physical properties etc.
- cause of mutations
- types of reactions which can be modified or controlled genetically.
  - vital modifications of metabolism alkaptonuria etc
  - non-vital modifications such ~~pigme~~ skin pigments of rabbits

- genes have a position effect
  - dominant gene - results in production of enzyme etc.
  - mutation of genes involves loss of a function - recessive genes

- general nature of action of genes
  - blocking of metabolic reactions
  - a way of arriving at actual number of chemical steps in a synthetic process.

- assume that all processes in cells are genetically controlled

1- Give titles and authors of 5 reference books you have found useful in this course.

2- What are the distinguishing differences between fermentation and respiration?

3- Give a scheme for the oxidation and resynthesis of glycogen, in animal tissues, giving names or formulae of intermediate products.

4- What do the following have in common in structure and function?

B_1 B_2 nicotinic amide pyocyanine

5- Discuss very briefly the significance of nucleotides in biological processes.

6-. State very briefly the main scientific contributions of:

- a. Harden
- b. Neuberg
- c. Warburg
- d. Wieland
- e. Szent Gyorgyi
- f. Meyerhof
- g. Parnas
- h. Krebs
- i. Kluyver
- j. Scott-Moncrieff
- k. Lwoff
- l. Beadle

(as considered in this course)

7- Is there any relationship between the following, if so, what?

- a. Phosphorylase and cytochrome
- b. Alkaptonuria and alkalosis
- c. B_1 and acetic acid
- d. Succinic and keto-glutaric acids
- e. Symbiotic bacteria and carnivorous animals
- f. Cytochrome and alcoholic fermentation

8- Is an understanding of the principles of heredity of any value to a biochemist? If so, what or why?

9- List 5 points of similarity between viruses, phages, and genes.

10- In the final analysis what determines the characteristics of a given species of plant or animal?

11- What is your frank opinion of this course? Any suggestions? (Answer not to be counted in grade unless too obviously laudatory, and then only negatively) v

Comparative Biochemistry - Chem 205

1/7/40)

Baldwin - Comparative Biochem

General: - Perspectives in Biochem - Cambridge
- Stephenson
- Pirie
- Baldwin

Comparative Biochem is concerned with the similarities of the underlying principles of biochem.

Defn. or properties of the living: -
- cell structure (most quest. as to whether cell structure is necessary - virus?)
- metabolism (again possible exception is virus).
- growth + reproduction
- motion
- adaption to environment

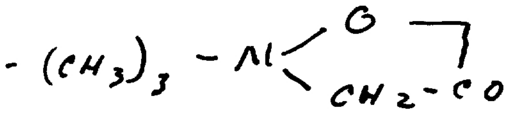
These are some of the criteria of life - but all are not necessary or fundamental.

Production of energy
Utilization of energy
Regulation of processes of ~~our~~ living organism.

Biochemical systems at various evolutionary levels.
1. Inorganic chemicals
a Blood system :- supposedly similar to sea water. Thought perhaps living organisms come first from sea.

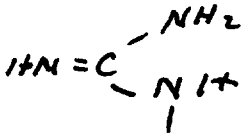
2. Higher organic chemicals - wide spread thru out various living organisms.

- NH_3, trimethyl amine,
- $(CH_3)_3 N=O$ - excretory material
- $(CH_3)_4 N-OH$ - found in jelly fish



$-(CH_3)_3-N\langle{}^{O}_{CH_2-CO}$

- nicotinic acid deriv.
- ~~[illegible]~~ guanidine deriv.



$HN=C\langle{}^{NH_2}_{NH}$

The purpose of these higher organic compds is usually very much the same different organisms.

Morphology is a representation of chemical structure.

"Recapitulation theory"

- Holds from chemical point of view of embryo
  - first excretes NH_3, then later the more complex compd's, urea etc.

Understanding of life best understood thru study of biophysical + biochemical processes of organism.

First undertake the most basic + fundamental processes - thus in all

1. Obtaining of energy
   - oxidation, dehydrogenation
2. Utilization of energy,
   - maintenance of order
   - transformation by way of syntheses
3. Control + Transmission of these separate, distinct synthetic processes.
   - N metabolism + synthesis

Biological processes can be attached from a chemical view point.

1/9/41) General Reviews:-

Oppenheimer - Handbuch. 2nd Ed. Vol II p 443-

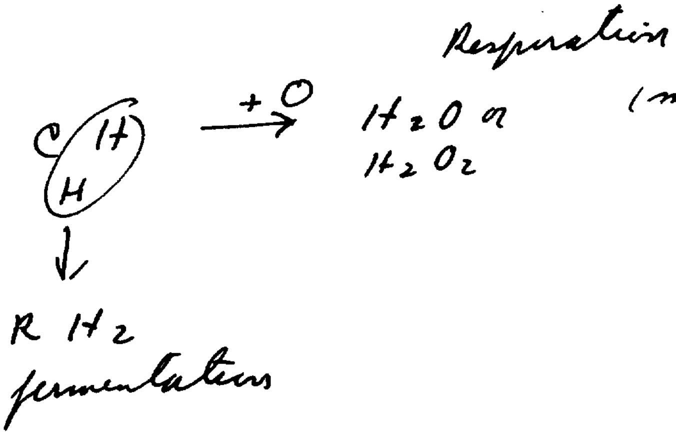
Neuberg - J. Bacti., <u>28</u>, 461 (34)

Harden - Alcoholic Fermentation
chapters on Mechanism.

2 ways of oxidation for production of energy.

(1) - with oxygen - respiration

(2) - without oxygen - fermentation



(more complete than fermentation)

Respiration is much more efficient manner of obtaining energy than fermentation

The first mechanism for production of energy was probably anaerobic (in sea water)

Cite bacteria as example

- Anaerobic organisms
- Facultative anaerobic organisms
- Those preferring aerobic mechanism but can live in anaerobic conditions (yeast).
- Aerobic organisms such as molds.

B. Coli - aerobically

$$CH_2 + O_2 \longrightarrow H_2O + CO_2$$

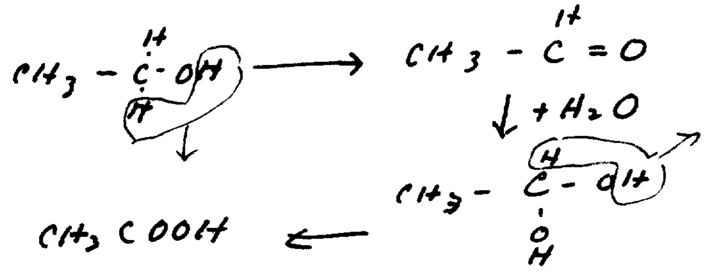
$$\text{Glucose} \xrightarrow{\text{anaerobic}} \text{nothing}$$

but $\text{Glucose} \xrightarrow[NaNO_3]{\text{anaerobic}}$ can carry out anaerobic ferment.

Acetic acid Bact.

thought that: $EtOH \xrightarrow[\text{aerobic}]{O}$ Acetic acid

Actually (Wieland)



$$CH_3-\overset{H}{\underset{H}{C}}-OH \longrightarrow CH_3-\overset{H}{C}=O \xrightarrow{+H_2O} CH_3-\overset{H}{\underset{OH}{C}}-OH \longrightarrow CH_3COOH$$

(OVER)

O_2 not necessary to accept 2 H first removed from C_2H_5-OH activation of H's & then

It is essentially an dehydrogenation rather than oxidation since Methylene blue will accept the H's in this bacterial oxidation of EtOH to acetic acid (Wieland)

$$M.B. + H \rightarrow M.B H$$

Specialization was little possible until respiratory oxidative mechanism replaced fermentation mechanism (in scale of evolution)

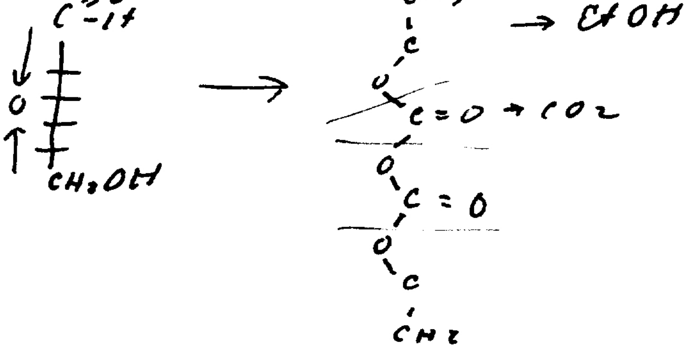
Best to study oxidative or fermentative mechanisms in one celled organisms.

Mechanisms of anaerobic fermentations

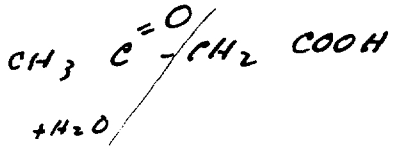
(1) - Alcoholic fermentation

$$\text{Hexose } (C_6H_{12}O_6) \rightarrow 2CO_2 + 2C_2H_5OH$$

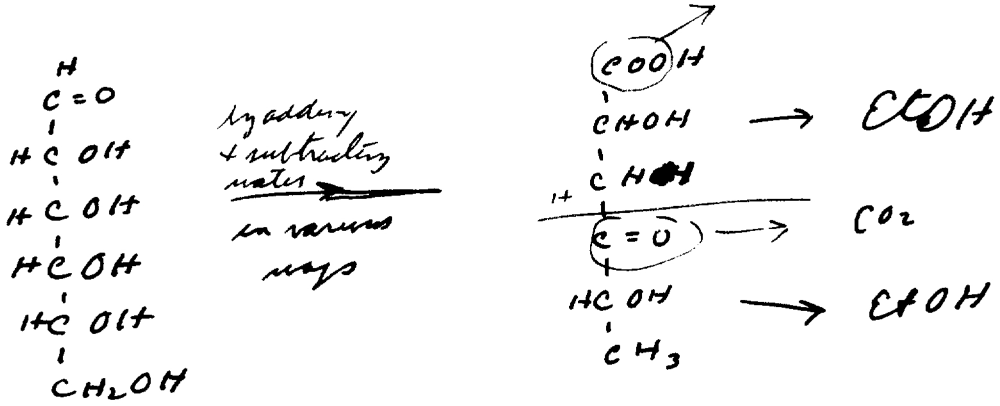
First scheme for this reaction :- Baeyer 1870



another scheme :–



$CH_3\,\overset{=O}{C}\,/\,CH_2\,COOH$ $\underset{+H_2O}{}$ → 2 molecules of acetic acid.

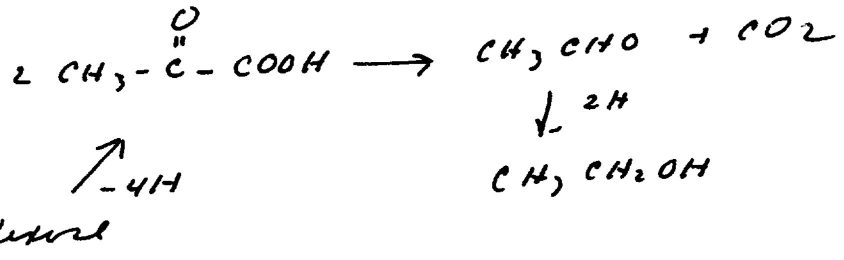


etc ! ????

Neuberg 1914 – established chemical basis of alcoholic fermentation

1902 – Magnus Levy – CH_3CHO basis for alcoholic ferment.

1910 – Kostychew pyruvic acid " "



$$2\ CH_3-\overset{O}{\overset{\|}{C}}-COOH \longrightarrow CH_3CHO + CO_2$$

$$\xrightarrow{2H} CH_3CH_2OH$$

$$\text{Hexose} \xrightarrow{-4H}$$

These schemes don't explain presence of glycerol, HAc etc found in alcoholic fermentation

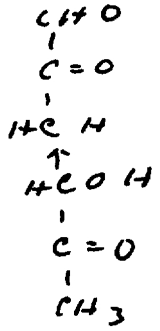
Neuberg 1914 – accepted pyruvic acid as an intermediate in alcoholic fermentation

(OVER)

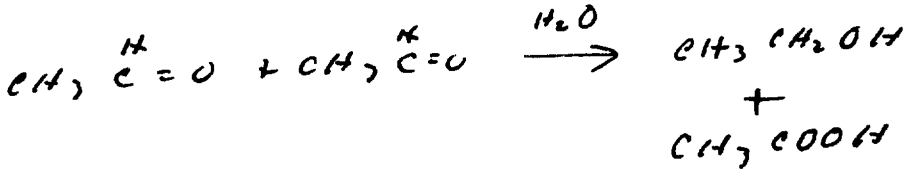
- Found $CH_3\overset{O}{\overset{\|}{C}}-CHO$ intermediate

Mass yeast culture → methyl glyoxal

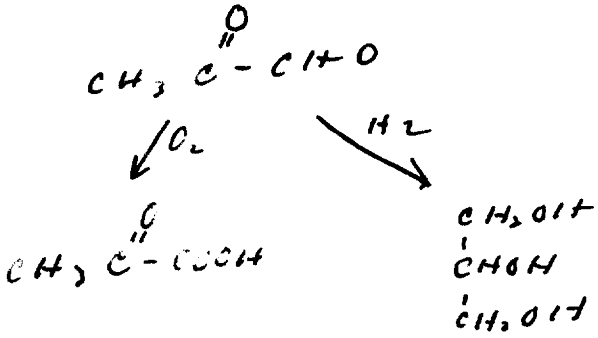
Ferment ⟶ lactic acid.
(glyoxalase)



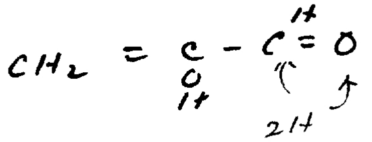
Neuberg also considered possibility of Cannizzaro reaction :-



$$CH_3\overset{H}{C}=O + CH_3\overset{H}{C}=O \xrightarrow{H_2O} CH_3CH_2OH + CH_3COOH$$



Comes to cell with ~~phenolic~~ to enolic form.



So Neuberg formulated as follows the alcoholic fermentation :-

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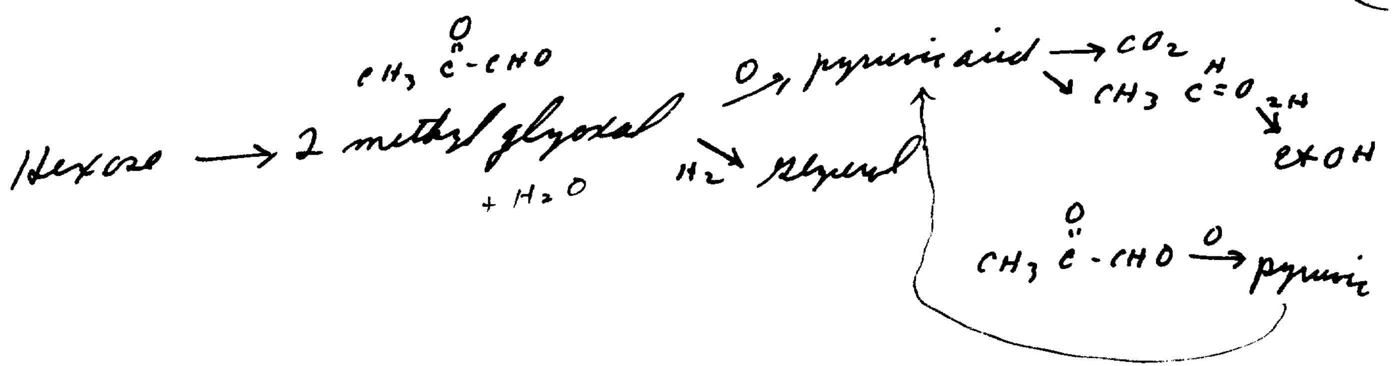

Remove acetaldehyde with $NaHSO_3$ - results in an accumulation of glycerol.

Hexose $\xrightarrow{NaHSO_3}$ 1 acetaldehyde + 1 CO_2 + 1 glycerol.

Can also remove CH_3CHO by adsorption by charcoal.

Hexose $\xrightarrow{NaOH}$ 1 glycerol. → CO_2 + ½ HAc + ½ EtOH

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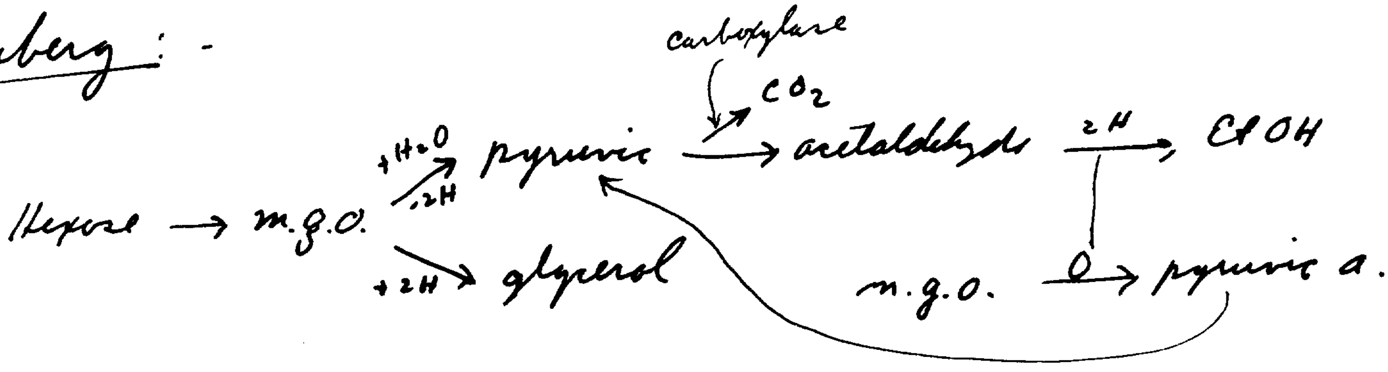
Harden - (Biol libr.) Stephenson - Bact. Met. 77-87
Oppenheimer - Stern - 244-249

Neuberg: -



Carboxylase enzyme
Glyoxalase $\xrightarrow{m.g.o.}$ lactic acid

Hex. $\rightarrow$ m.g.o $\xrightarrow{\text{glyoxalase}}$ lactic a.

Lehmann :- glutathione necessary as co-enzyme

Where does lactic acid come from in muscle? :-

Acetaldehyde $\xrightarrow{2H}$ EtOH

Meyerhof proposed :-

Pyruvic $\xrightarrow{2H}$ lactic

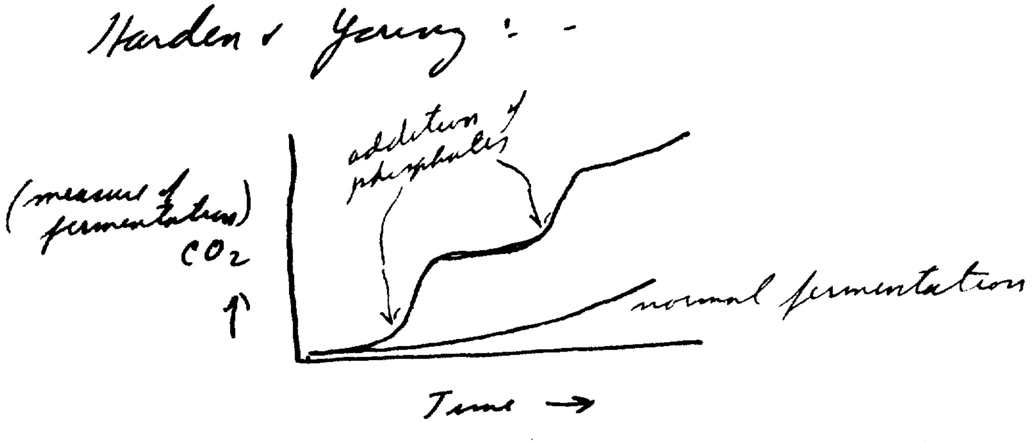
∴ picture is :- (in muscle)

Hexose $\overset{O}{\cdots\rightarrow}$ pyruvic a. $\xrightarrow{2H}$ lactic a.

— o —

Relation of Phosphates to alcoholic fermentation :-

Harden & Young :-



Found that hexose phosphates formed.

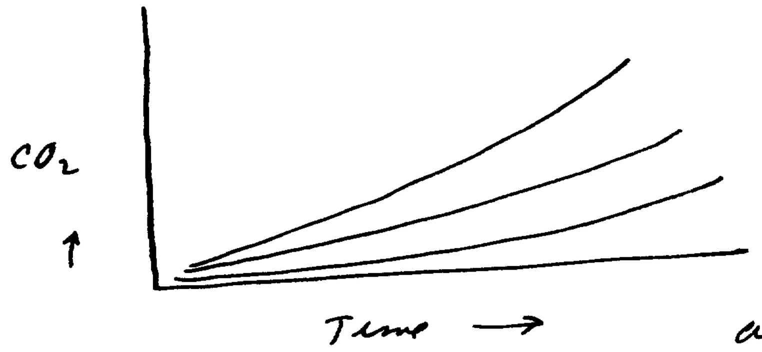
$$2\ \text{Hexose} \xrightarrow{+2PO_4} 2CO_2 + EtOH \rightarrow 1\ \text{Hex. diphosphate}$$

$$\downarrow$$

$$2H_3PO_4 + 1\ \text{activated hexose}$$

Co enzyme of zymase in normal serum:-

Harden:-



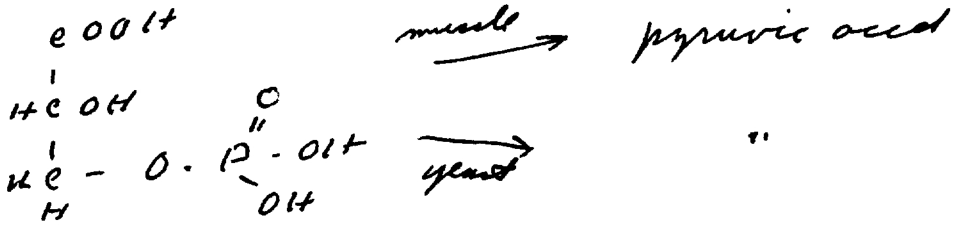
Drop in activity due to inactivation of co-zymase. Found that normal activity restored by introduction of normal serum which has co-zymase present.

Back to muscle metabolism:-

Meyerhof:- postulated & proved intermediate nature of pyruvic a. in muscle metabolism.

m.g.o. $\not\rightarrow$ pyruvic
lactic $\not\rightarrow$ "
Hexose phosphate → "
or glyc.

Nelson :- isolated 3·P- glyceric acid

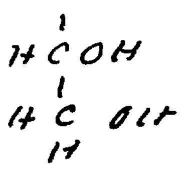


$$\begin{array}{l} COOH \\ \;| \\ HCOH \\ \;| \\ HC - O \cdot \overset{O}{\overset{\|}{P}} - OH \\ H \qquad\quad \diagdown OH \end{array} \quad \begin{array}{l} \xrightarrow{\text{muscle}} \text{pyruvic acid} \\ \xrightarrow[\text{yeast}]{} \text{"} \end{array}$$

Lohmann :- isolated from muscle (with NaF) - also a mixture cont. 3-phospho-glyceric acid.

$$\begin{tikzcd}
 & & & \alpha\text{ glycerol P} \\
\therefore\ \text{Hexose} \arrow[r] & \text{Hexose diphosphate} \arrow[urr, "2H"] \arrow[drr, "\text{oxidation}"] & & \\
\text{EtOH} & \text{CO}_2 \arrow[l] & & \text{3-phospho glyceric acid} \arrow[dl] \\
 & & \text{pyruvic a.} \arrow[ul, "\text{in yeast}"] \arrow[dll, "2H"', "\text{in muscle}"] & \\
\text{lactic a.} & & &
\end{tikzcd}$$

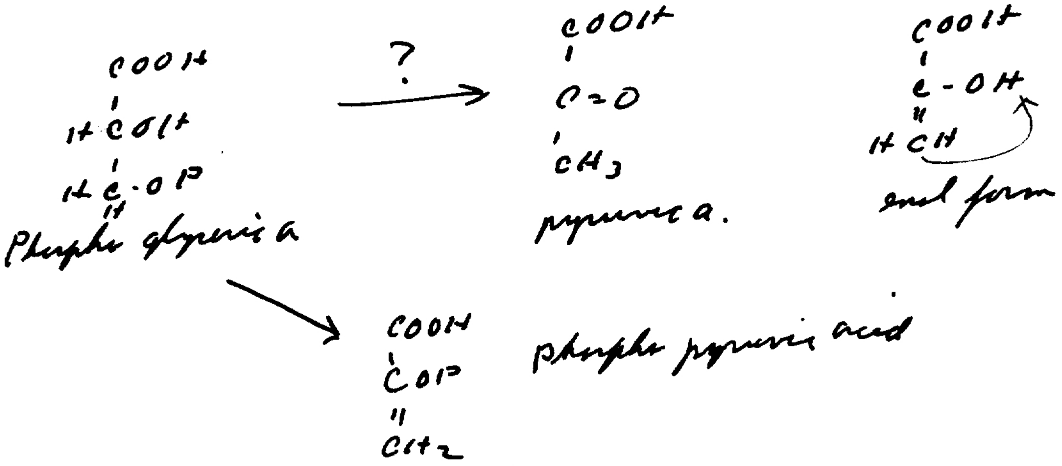
Looked for reduced product upon oxidation of Hexose di-phosphate to 3-P-glyceric acid:- - found to be α - glycerol P.



$$\begin{array}{l} CH_2OP \\ \;| \\ HCOH \\ \;| \\ HC\ OH \\ H \end{array}$$

. Lohmans ester.

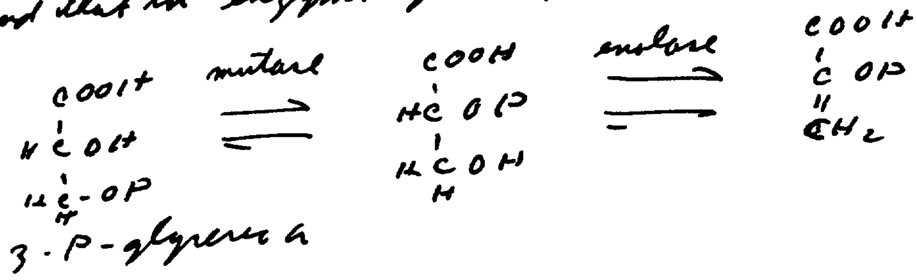
$$\begin{tikzcd}
\text{Hexose} \arrow[r] & \text{Hex. di P. (triose P)} \arrow[r, "O"] \arrow[dr, "2H"] & \text{3-phospho glyceric acid} \arrow[r, "mus."] & \text{pyruvic a.} \arrow[r, "2H"] & \text{lactic} \\
& & \alpha\text{-glycerol P} \arrow[r] & \text{Triose P} \arrow[ull, bend left] &
\end{tikzcd}$$



$$\underset{\text{Phospho glyceric a}}{\begin{matrix}COOH\\ |\\ HCOH\\ |\\ H\overset{}{C}\cdot OP\\ H\end{matrix}} \xrightarrow{?} \underset{\text{pyruvic a.}}{\begin{matrix}COOH\\ |\\ C=O\\ |\\ CH_3\end{matrix}} \qquad \underset{\text{enol form}}{\begin{matrix}COOH\\ |\\ C-OH\\ \|\\ HCH\end{matrix}}$$

$$\searrow \underset{\text{phospho pyruvic acid}}{\begin{matrix}COOH\\ |\\ COP\\ \|\\ CH_2\end{matrix}}$$

Found that in enzyme system:-



$$\underset{\text{3-P-glyceric a}}{\begin{matrix}COOH\\ |\\ HCOH\\ |\\ HC-OP\\ H\end{matrix}} \overset{\text{mutase}}{\rightleftharpoons} \begin{matrix}COOH\\ |\\ HC\,OP\\ |\\ HCOH\\ H\end{matrix} \overset{\text{enolase}}{\rightleftharpoons} \begin{matrix}COOH\\ |\\ C\,OP\\ \|\\ CH_2\end{matrix}$$

Using P-pyruvic acid in fermentation process:-

y
m P-pyruvic a. ↛ pyruvic acid

Pyruvic $\xrightarrow{2H}$ lactic a.
+
p-glycerol → triose P.

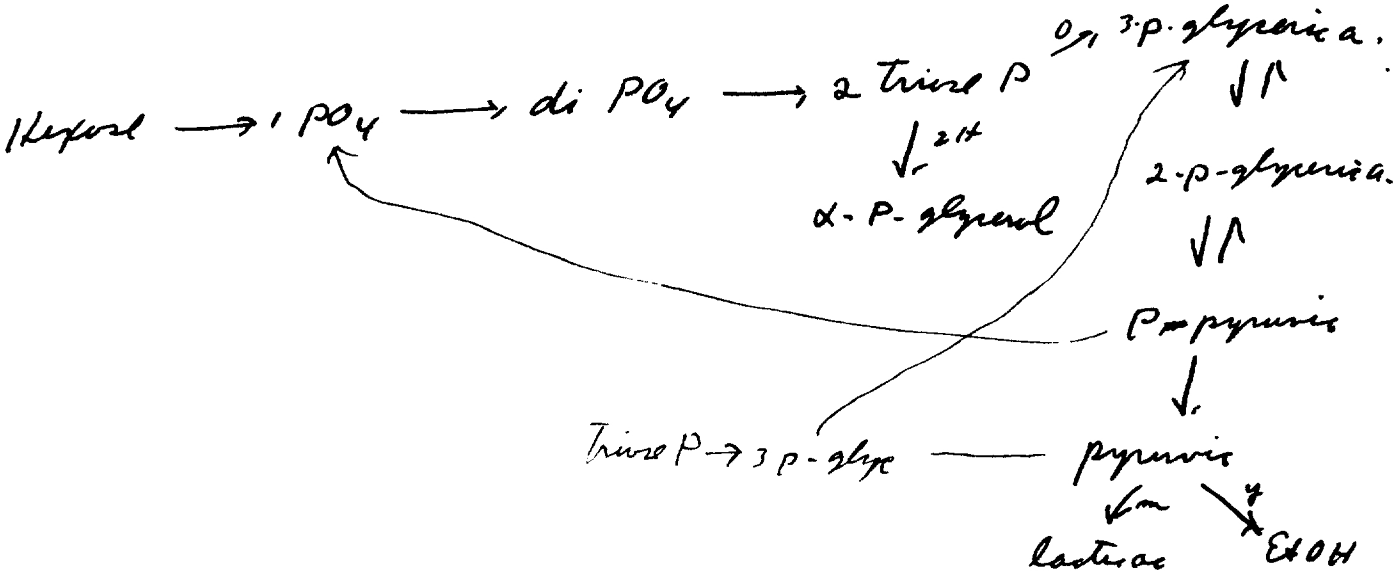
} not take place in normal prepns. but these does react:-
Pyruvic$^{(m)}$ + Hex di P $\xrightarrow{NaF}$ lactic a + p. glyceric a.
acetald$^{(y)}$ + " → EtOH + p-glyceric a

// hexose added:-

Hex. + PO₄-pyruvic → pyruvic a + Hex. di P.
(or P-glyceric a.)

Proves that PO₄ transferred readily to Hex di P.

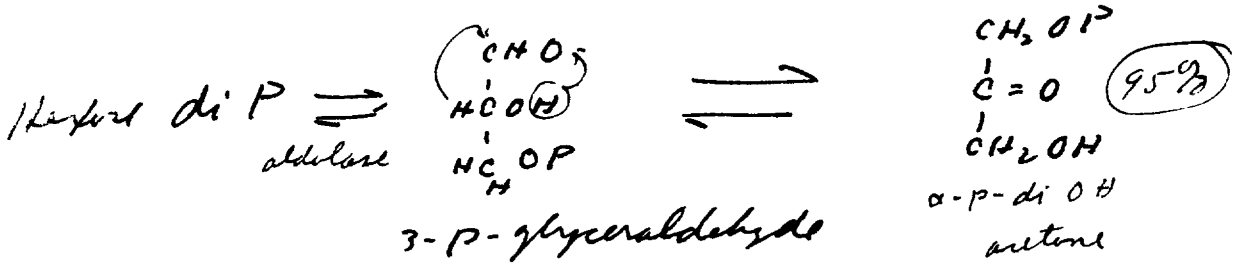
Final scheme in either yeast or muscle :-



Iodoacetic acid inhibits reaction:-

$$2 \text{ triose P} \xrightarrow{O} 3\text{P-glyceric a.}$$

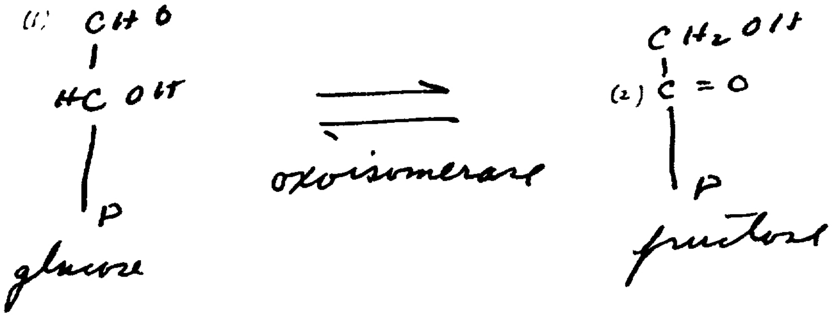
$$\xrightarrow{2H} \alpha\text{-P-glycerol.}$$



\+

α-P-di OH acetone

— o —



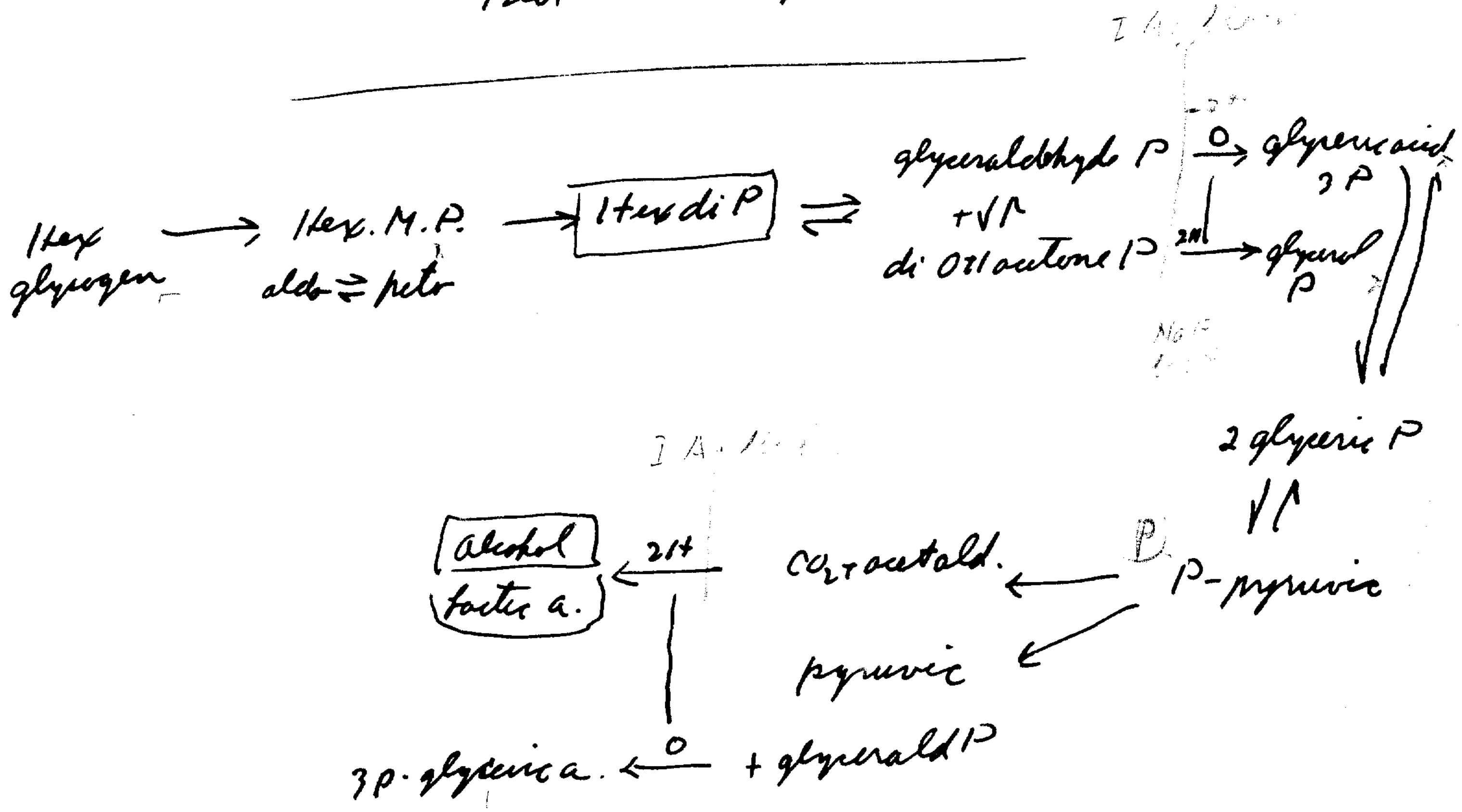
8.

1/16/40

Coenzymes (Review)
Baumann + Stare
Physiol. Rev. (1939) p353

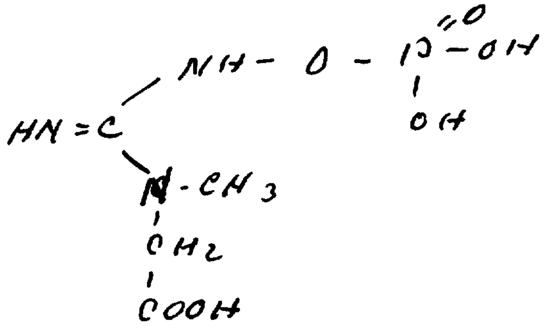
Co-enz. alcoholic ferm.
Stephenson - Bact. Metab
- muscle & energetics
Perspect. Biochem - Needham

Review - fermentation as whole.
Oppenheimer & Stern
Biol Oxid p 241



In muscular contraction - energy is supplied by production of lactic acid.

Egeloff -1927 - discovered phosphagen or phosphocreatine in muscle



H_3PO_4 is split off during muscular contraction

$C \rightleftharpoons CP$

$+ P$

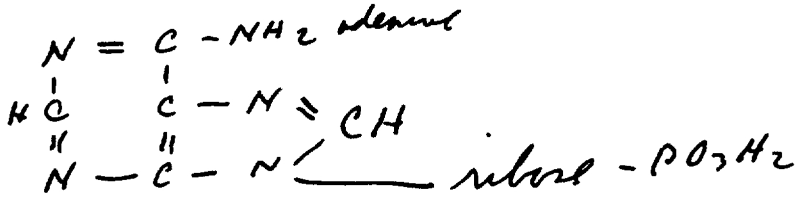
$+11,000$ cal/mol
energy liberated

Creatine + P-pyruvic acid → no reaction

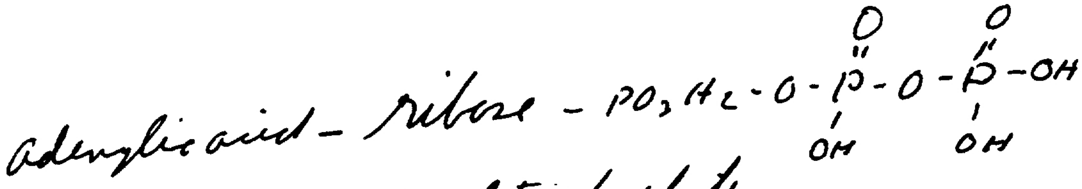
Creatine phosphate + Hex → no reaction

Coenzyme of phosphorolation + dephosphorolation is necessary.

Adenylic acid was found to be this co-enzyme



adenylic acid.



$$\text{adenylic acid - ribose} - PO_3H_2-O-\overset{O}{\underset{OH}{\overset{\|}{P}}}-O-\overset{O}{\underset{OH}{\overset{\|}{P}}}-OH$$

adenyl triphosphate
or pyro phosphate

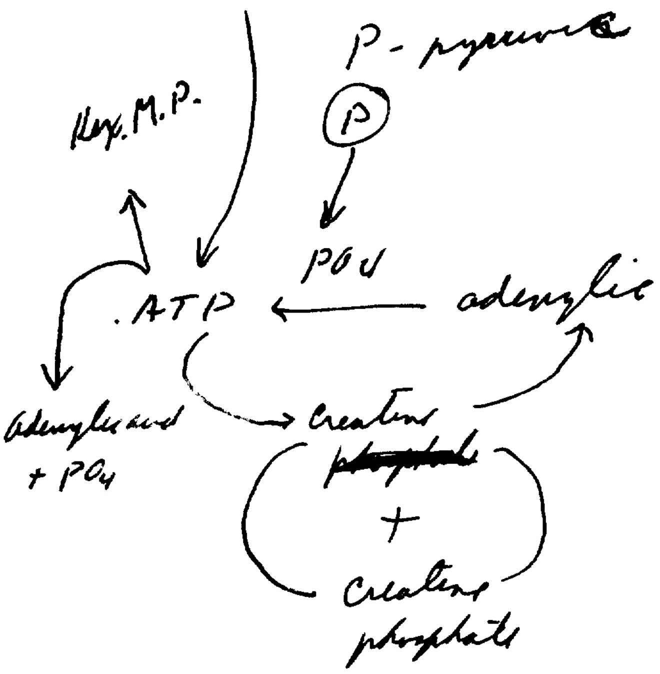
adenylic acid + pyro P → $\underbrace{\text{adenyl tri phosphate}}$

This compound does give phosphate to glycogen or hexose

C.Posphate + Adenylic acid + Hexose → Hexosephosphate

Steps of phosphate transfer scheme:-

PO_4 (inorganic) from reaction $\underset{3P}{\text{glyceric a.}} \rightleftharpoons$ 2 glyceric P



ATP = adenylic triphosphate

$$A + CP \rightleftharpoons ATP + C$$

So in equilibrium thermodynamically

Creatine phosphate is store house of phosphate in phosphoralation mechanism

Co-phosphorylase

Co-zymase.

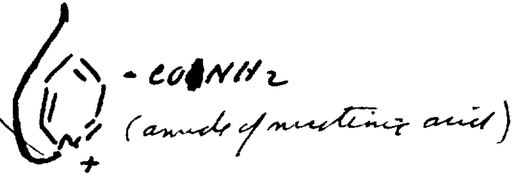
Warburg :-

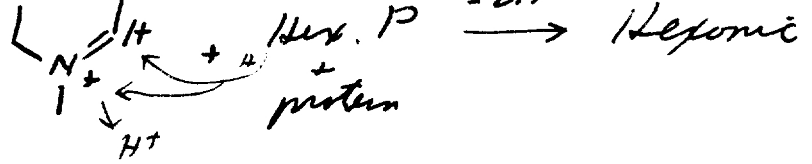
$$\text{Hex. mono P} \xrightarrow[\text{(dehyd. } -2H)]{O} \text{P-hexonic acid}$$

To make this reaction possible :-

$$\begin{array}{l}\text{Hex mono P}\\ \quad + \\ \text{protein}\\ \quad + \\ \text{co-enzyme}\end{array} \xrightarrow[\text{(dehyd. } -2H)]{O} \text{P. hexonic acid.}$$

This coenzyme was:- adenine - ribose - phosphate
|
ribose - phosphate — phosphate





This is known as Warburg's coenzyme II

Coenzyme I (cozymase) = Di P nucleotide

Coenzyme I + protein A = alcohol dehydrogenase

Coenzyme H_2 + acetaldehyde → Co-enz oxidized + EtOH
(reduced co-enz.)

" + pyruvic → " + lactic

These reactions take place stoichiometrically

—o—

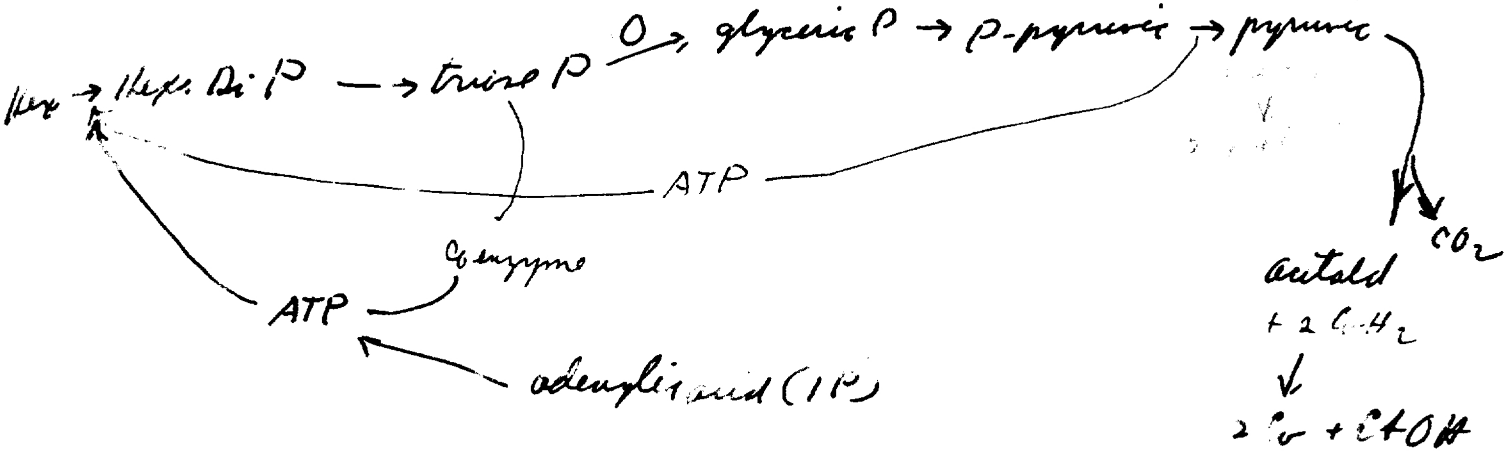
Needham & Meyerhof :-

2 triose P + a Coenzyme + 2 H_3PO_4 + adenylic acid

↓

2 Coenzyme H_2 (reduced) + ATP + 2 glyceric acid

Complete scheme of decomp of Hex Di P. :-

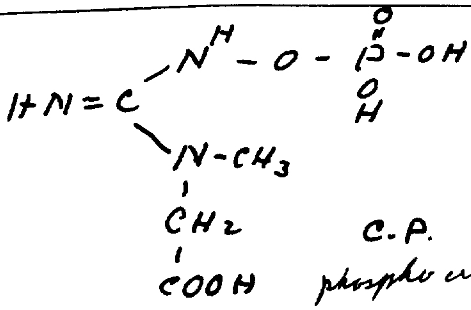


1/21/41

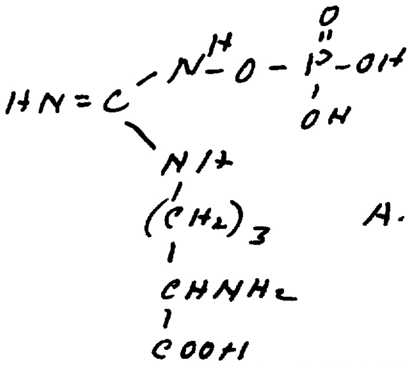
Stephenson pp 87-110

Kluyver - Chem. Act. Microorgs.

Baldwin - p 66.



C.P. phospho creatine



A.P.

	AP	CP	AP→ATP	ADP→ATP
Protozoa	−	−		
Porifera	−	−		
Coelenterata	+?	−?		
Annelida	+	−		
Arthropoda	+(enz)	−	+	
Mollusca	+	−	+	
Echinodermata - Asteroidea (l-noto)	+	−		
Holothuroidea -	+(enz)	−(no)		
Echinoidea -	+(enz) ±	± +		+
Chordata				
Proto " - Tunicata	+	−		
Enteropneusta (l) a-noto	+	+		
Cephalochordata	−	+		
Vertebrata	−(no)	+(enz)		+

Phosphorylation - review

3 ways -

(1) Inorganic esterification of glycogen

(2) Glycogen - AA system

(3) Breakdown of AA.

Co-enzymes

Diff. between y + m

y has true carboxylase

m has lactic dehydrogenase (?)

Phosphate cycles:- how valid are they?

- Hexose P isolated in plants
- Triose P " " "
- Speed up fermentation by addition of inorganic PO4
- Essential nature of AA system of co-enz. function
- Inorg. phosphate decrease during fermentation
- Phosphagen cycles

Neuberg's schemes – supported by
- glyoxalase activity
- necessity of glutathione

— o —

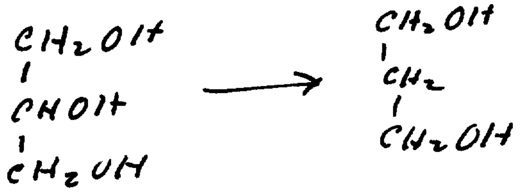
Therefore – 2 forms or courses of fermentation
(1) – Phosphorylated systems
(2) – The one with methyl glyoxal as intermediate (Neuberg).

— o —

Evolution of mechanisms :–
- first without enzymes
- more intermediates + enzymes
- several sources of PO_4 for phosphorylation

— o —

Bacterial – There is one species which can ferment glycerol.



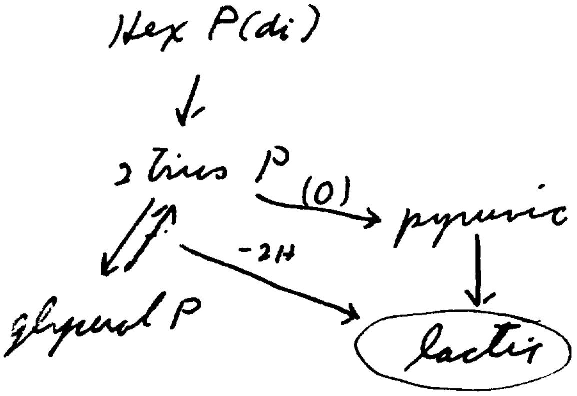
$$\begin{matrix} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{matrix} \longrightarrow \begin{matrix} CH_2OH \\ | \\ CH_2 \\ | \\ CH_2OH \end{matrix}$$

B. Coli :- fermentation of mannitol

	mannitol	glycerol	gluconic a.	saccharic acid
HAc	7	16	48	5.0
EtOH	27	16	2.6	1.0

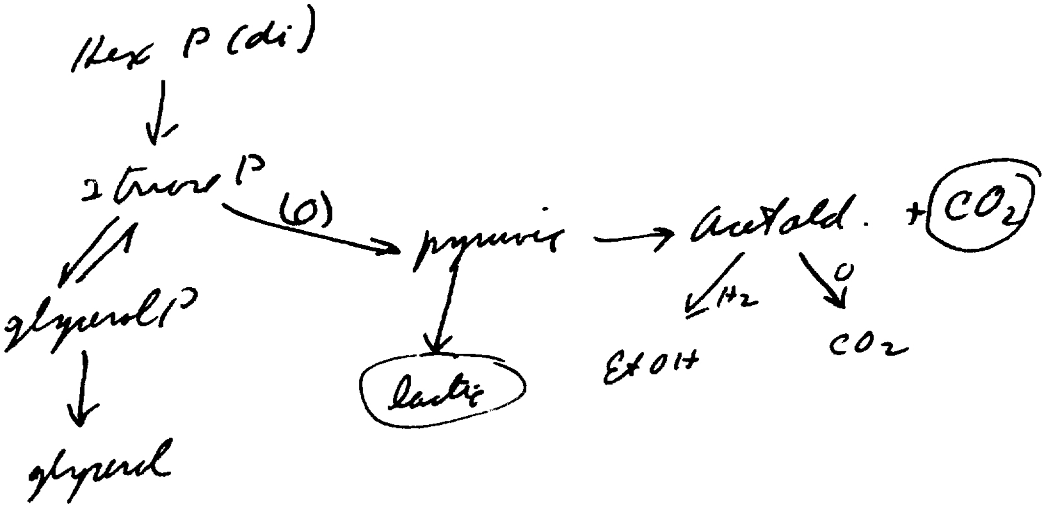
correlation bt ox v red. depending upon substrate

Simple bacterial fermentation :-



More complex system :-

EtOH, HAc, CO_2, lactic a. glycerol (end products)
1 : 1 : 1 80%

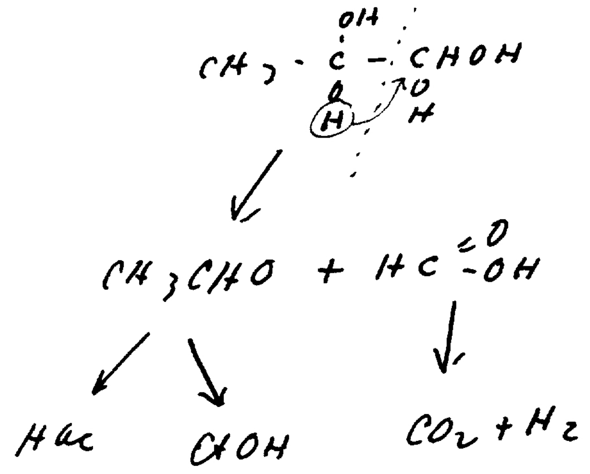


Bc. Coli Fermentation :-

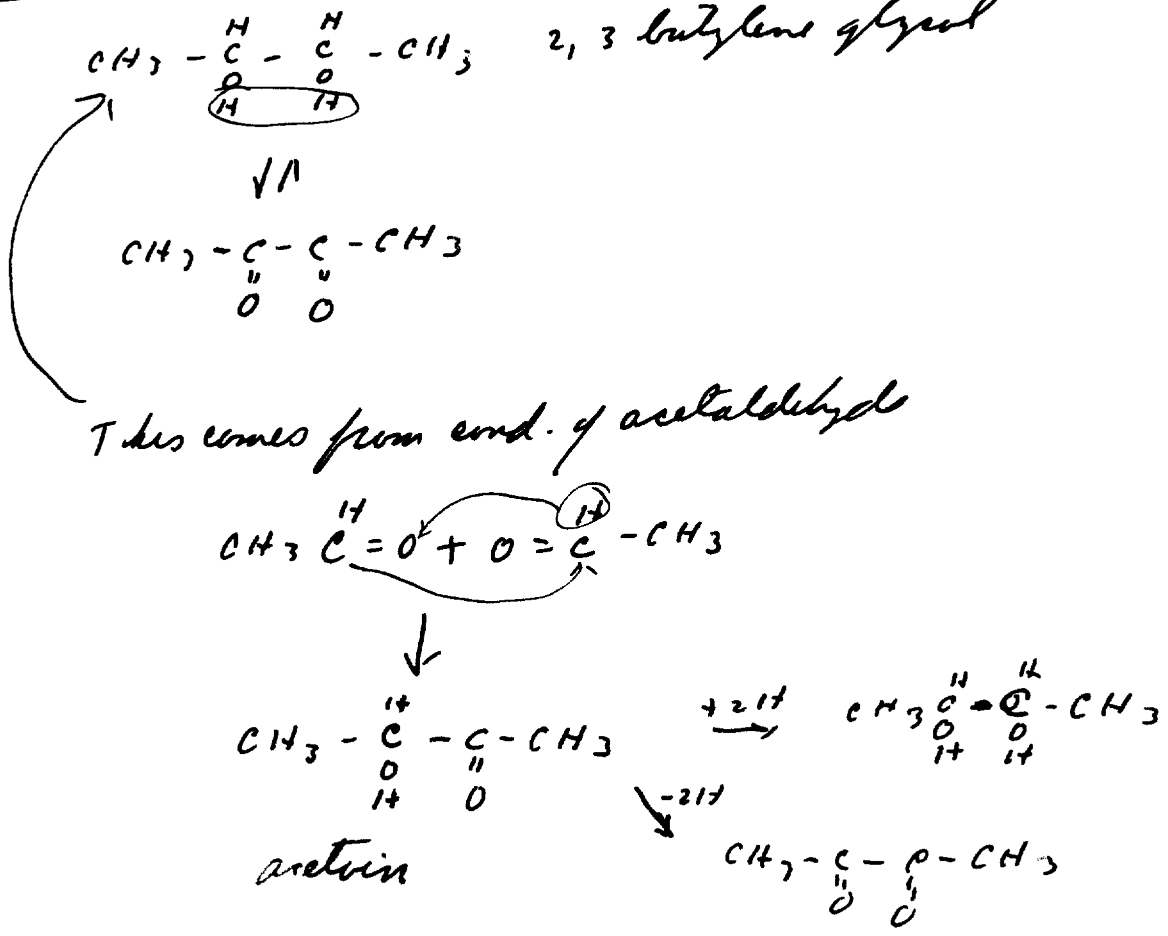
lactic acid - 50%
HAc 1 :
EtOH 1 } from acetaldehyde
H_2 : 1 :
CO_2 1 } from formic acid

(next page)

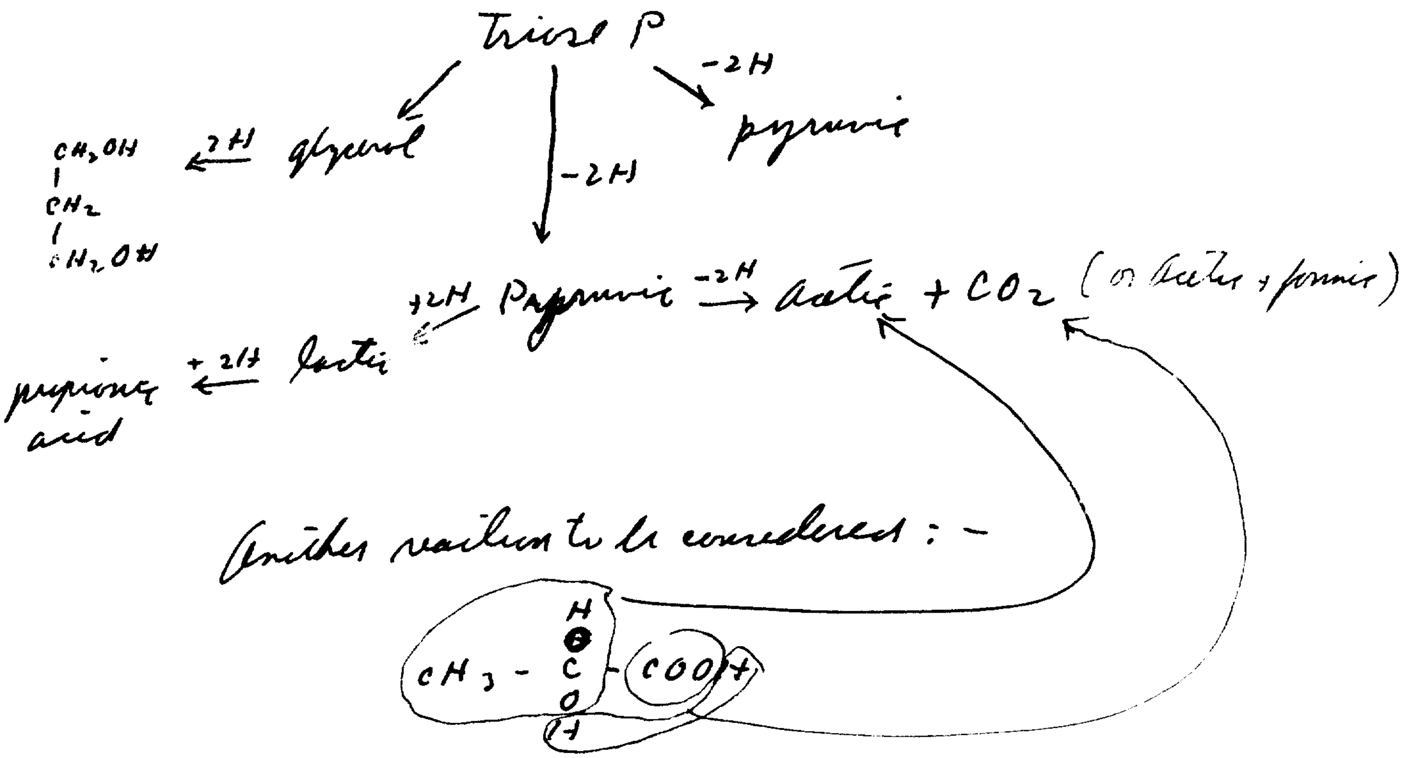
- lactic a. formed by traditional methods
- but the other triose phosphate molecule goes as follows :-



Another hexose breakdown :-



This comes from cond. of acetaldehyde



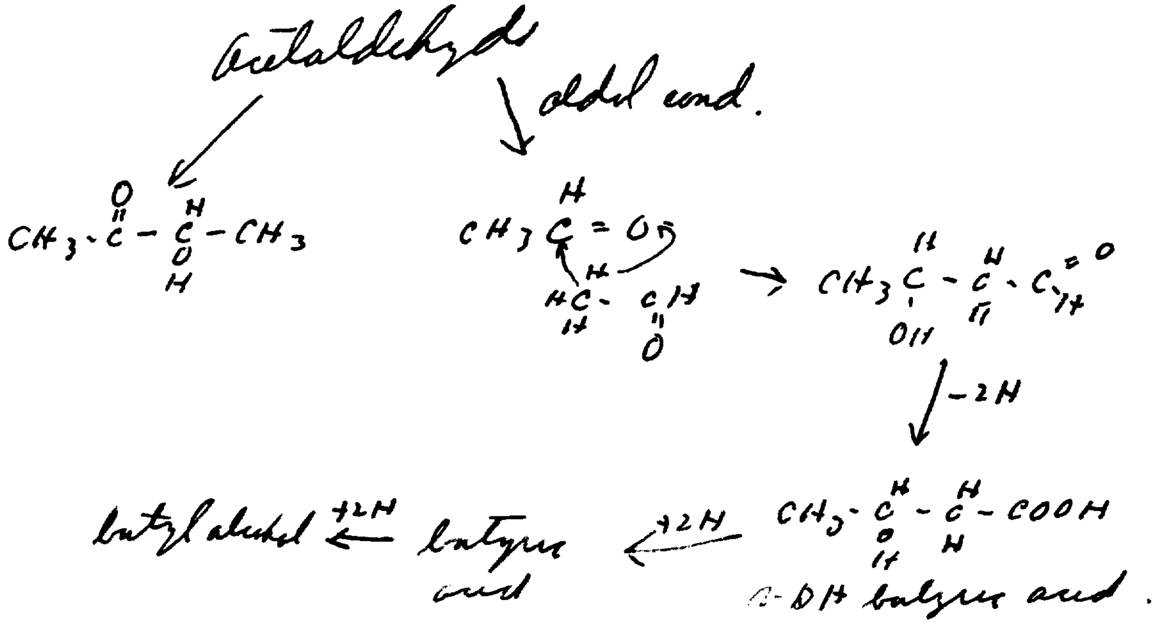
Another reaction to be considered:-

In butyric acid fermentation:-

butyric a. $\xrightarrow{+2H}$ butylalcohol

— o —

Condensation reactions:- (butyl alc. formation)

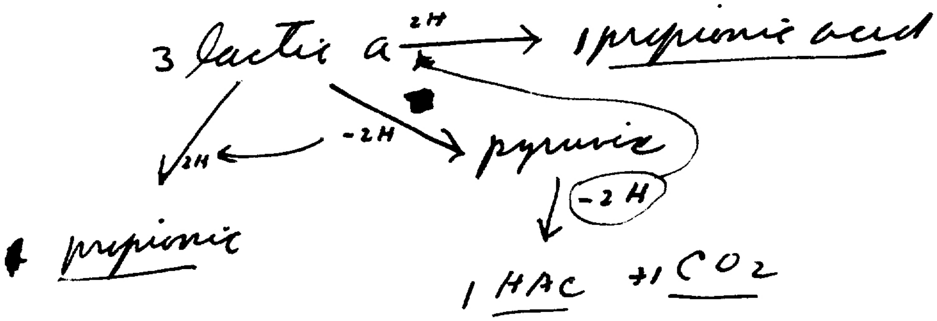


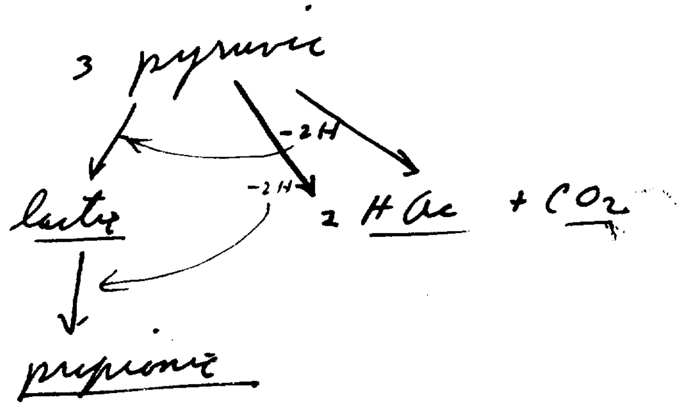
1/23/41

Stephenson p114 (Sorbose bact.)
Ann. Rev. Biochem (40) p29-33
(reactions pyruvic acid)
Biolog. Oxid. p.249-254
(general oxidative mech.)

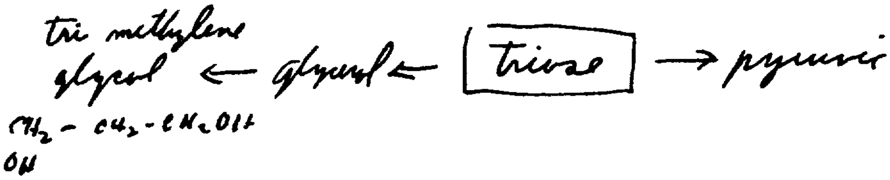
Significance of transitions between two phosphates (arginine + creatine)

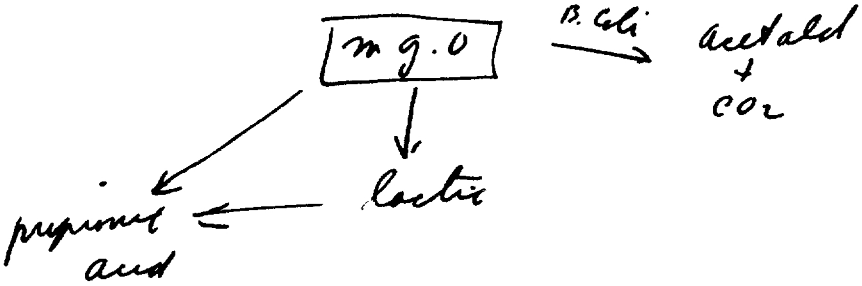
Propionic acid bacteria:-

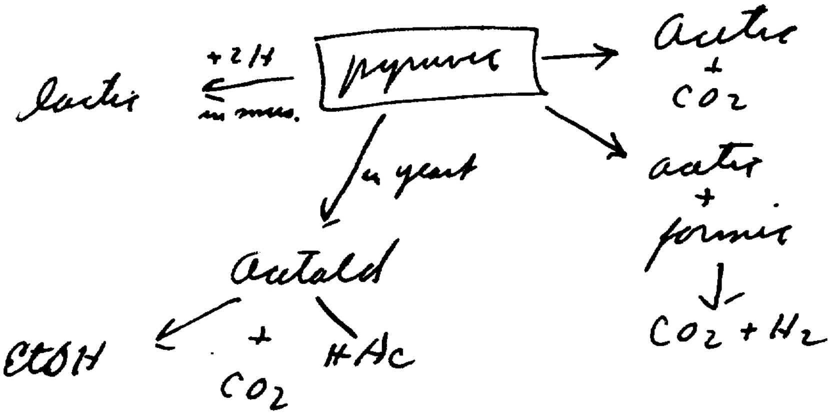




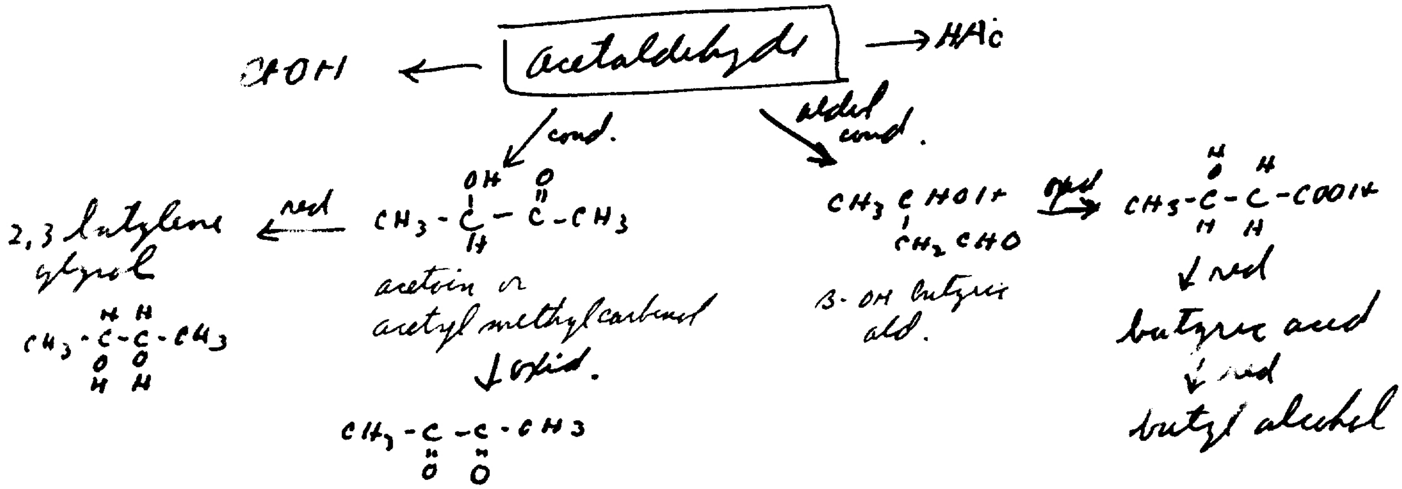
Anaerobic fermentation :-



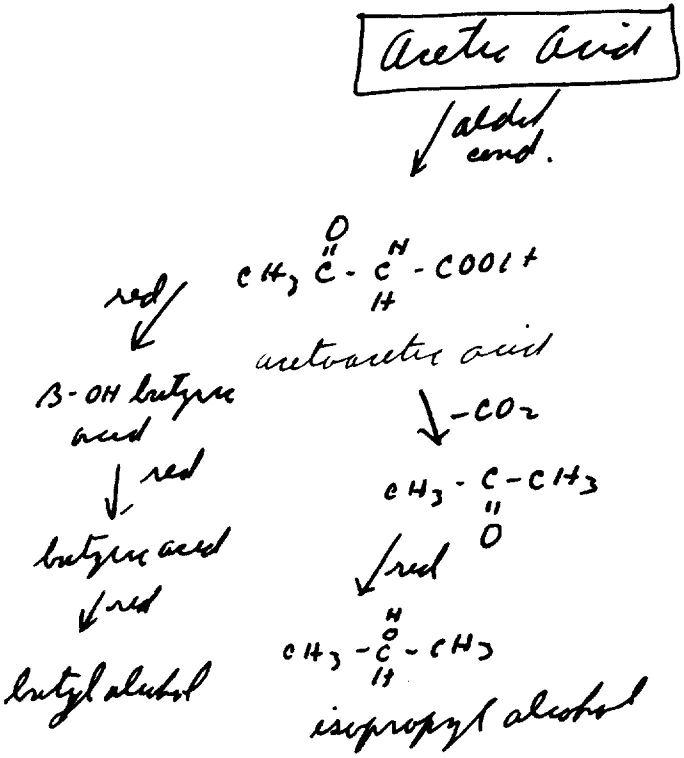




Possible reactions of acetaldehyde :-

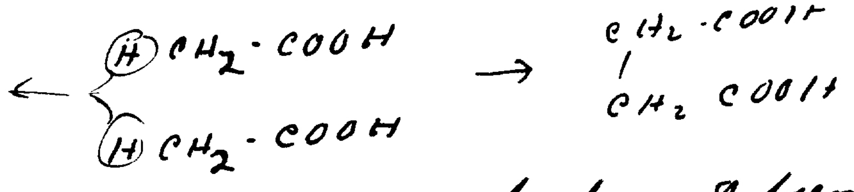


Possible reactions / acetic acid :- (anaerobic bacterial fermentation)



Several products which can't be explained by schemes developed from these :-

Succinic acid is one :-



$$\leftarrow \left\{ \begin{matrix} ⓗ\,CH_2\cdot COOH \\ ⓗ\,CH_2\cdot COOH \end{matrix} \right. \rightarrow \begin{matrix} CH_2\cdot COOH \\ | \\ CH_2\;COOH \end{matrix}$$

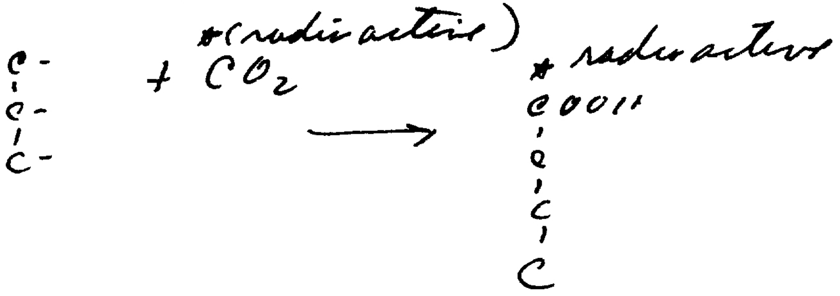
This mech. has not been proven

Another explanation :- not proven

$$\begin{tikzcd} & 2\,C \\ 6\,C\ \text{Hexose} \arrow[ur] \arrow[r] & 4\,C \arrow[r] & \text{succinic acid} \end{tikzcd}$$

(next page)

More schemes for succinic acid: - just a postulate



$$C-C-C + \overset{*}{C}O_2 \longrightarrow \overset{*}{C}OOH-C-C-C$$

Another unexplainable product - Methane: -

Possible scheme

$CO_2 \xrightarrow{4H} CH_4$

Since anaerobic - must have corresp. oxid.

EtOH → ~~Et~~ HAc.

Butyl → Butyric → acetic

— o —

In muscle 1/3 of lactic acid formed in muscle contraction is irreversibly + aerobically oxidized to $CO_2 + H_2O$ → releases energy. The other 2/3 lactic acid is resynthesized to glucose + glycogen.

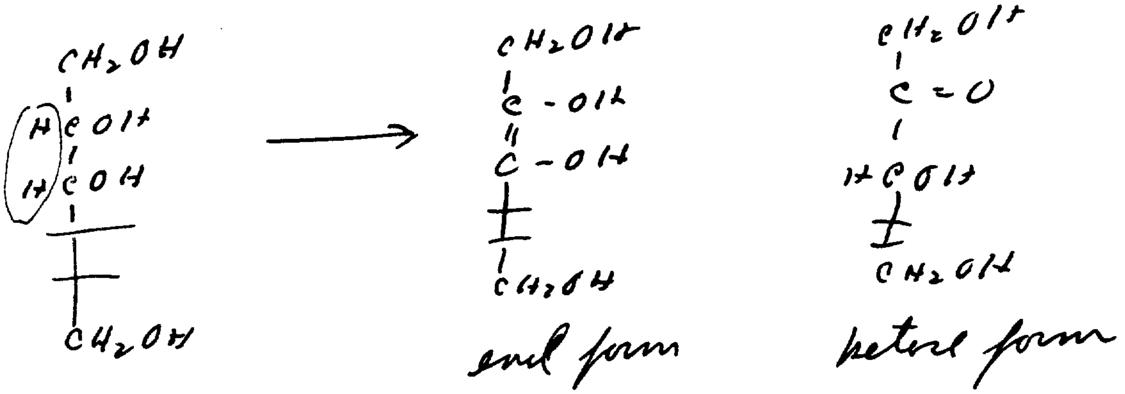
Sl. Gyorgyi: - 1/3 of lactic acid mol is oxidized

$$C_3H_6O_3 \rightarrow CO_2 + H_2O + (C_2H_4O_2)$$

↓ glucose, starch etc.

not 1 lactic a mol. out of 3.

Atypical Oxid. of Sorbose Bacteria:-



— o —

Aerobic Oxidations starting with pyruvic acid:-

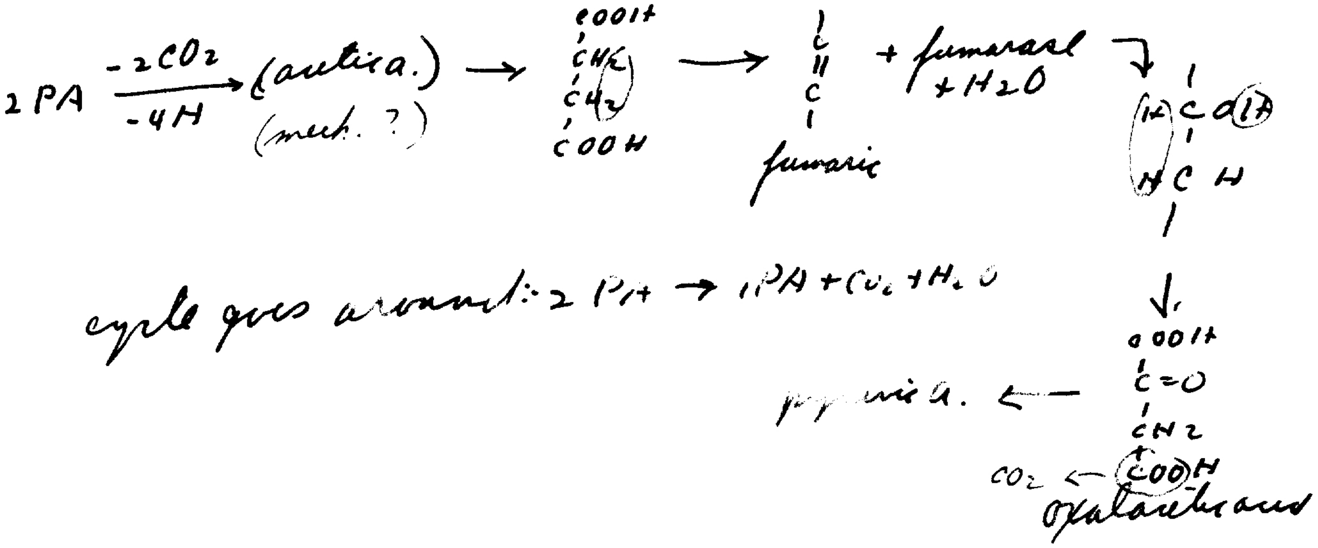
(1) pyruvic A $\xrightarrow{red}$ lactic gonococcus - anaerob.

PA $\xrightarrow{oxid}$ HAc + CO_2 brain - aerob.

PA $\xrightarrow{O_2}$ HAc + CO_2 - aerobic by either brain or gonococcus.

$\xrightarrow{O_2}$ $CO_2 + H_2O$ (complete breakdown)

Elliot - kidney chopped up. - complete breakdown of pyruvic acid



cycle goes around: 2 PA → 1PA + CO_2 + H_2O

Yeast :- when food is depleted - shown to be true

$$\text{EtOH} \rightarrow \text{Acet. ald.} \xrightarrow{2} \text{Acet a.} \rightarrow \text{Succinic acid}$$

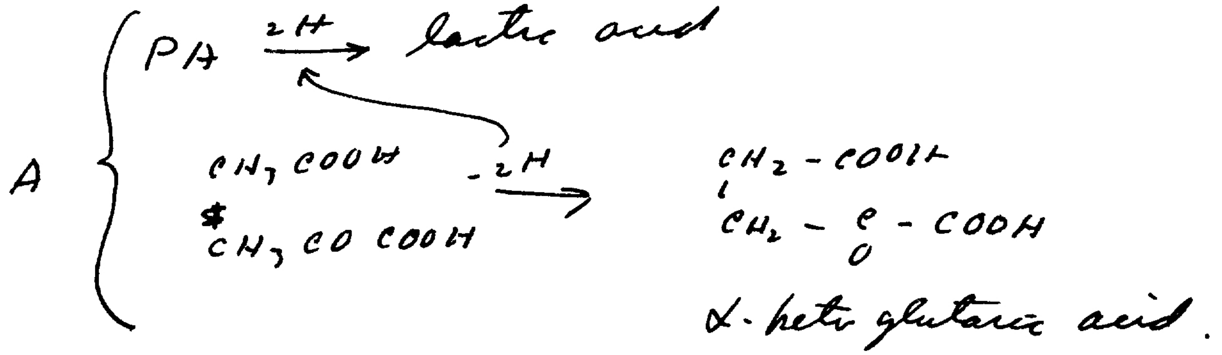
This fails to occur in animal tissues

Overall reaction of pyruvic a. to succinic a. does take place in animal tissue :-

$$2\,PA \longrightarrow \text{succinic a.} \longrightarrow 1\,PA + 3\,CO_2 + H_2O$$

Possible mechanism for prod. succinic acid in muscle:-

A

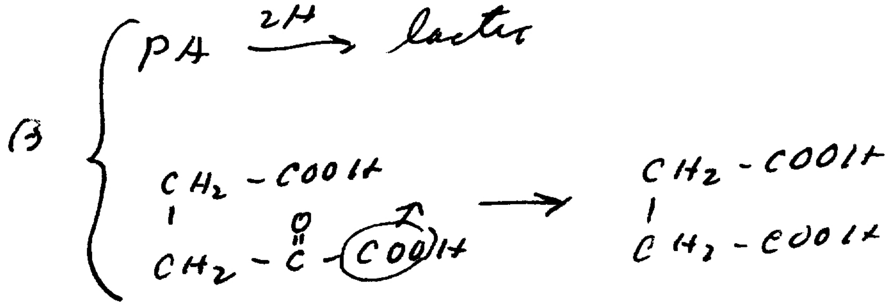


$$PA \xrightarrow{2H} \text{lactic acid}$$

$$CH_3COOH + CH_3COCOOH \xrightarrow{-2H} \begin{array}{l} CH_2-COOH \\ | \\ CH_2-\underset{O}{C}-COOH \end{array}$$

α-keto glutaric acid.

B



$$PA \xrightarrow{2H} \text{lactic}$$

$$\begin{array}{l} CH_2-COOH \\ | \\ CH_2-\overset{O}{\overset{\|}{C}}-\boxed{COO}H \end{array} \longrightarrow \begin{array}{l} CH_2-COOH \\ | \\ CH_2-COOH \end{array}$$

1/28/40

Vit. B_1 - di PO_4 is coenzyme of carboxylase

B_1 - di PO_4 — + protein A = carboxylase

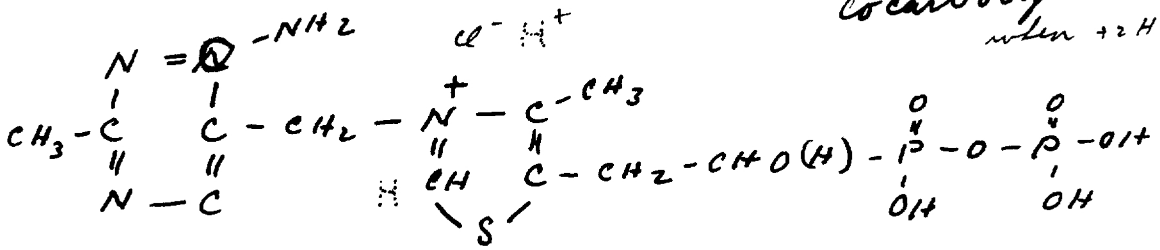
" — + " B = pyruvic dehydrogenase

— o —

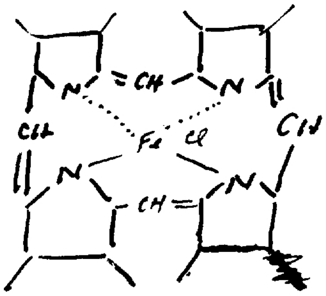
Barron - Physiol Rev '40, p184

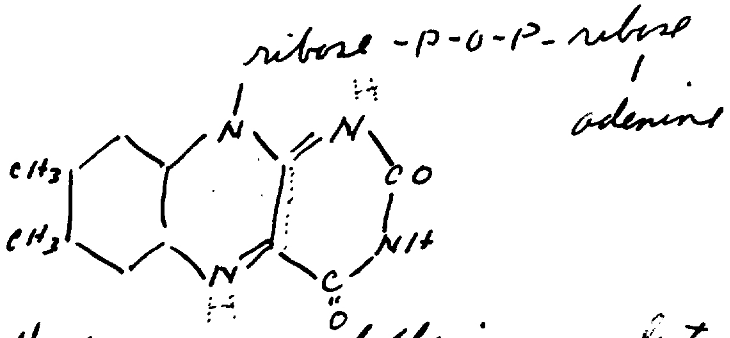
Biol Oxid. p 261-276

Ann. Rev. Biochem '40 p17-28



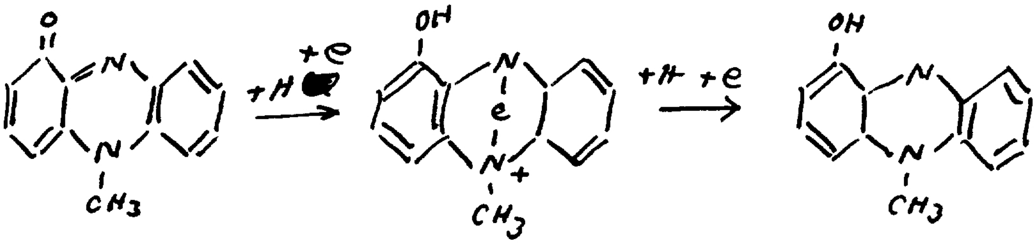
cocarboxylase
when +2H - pyruvic dehydrogenase





alloxazine riboflavin-nucleotide

pyocyanine



Thiamin or Vit B_1 deficiency - no respiration involving pyruvic acid. This corrected by addn of Vit B_1 to diet.

—

Ehrlich :-

1902 - Enzyme oxidizing aldehydes + reduction of Methylene blue

Wieland - dehydrogenation. activation of Hydrogens.

- Model systems: -

$$AH_2 + Pd \rightarrow PdH_2 + A$$

$$PdH_2 + O_2 \rightarrow H_2O_2 + Pd$$

$$+ M.B. \rightarrow M.B.H_2$$

This process carried out by all tissues

Thunberg 1925 :-

- washed tissue - anaerobic tissue no red. of M.B. unless H_2 donator added. If added, dehydrogenase enzymes found to be present

2 types of oxidation enzymes

1. Oxidases or aerobic dehydrogenases
2. Anaerobic dehydrogenases

(1). Oxidases - form H_2O_2 - this oxygen utilizable thru peroxidases for further oxidation

Coenzymes :-

- actually constituents of final enzyme

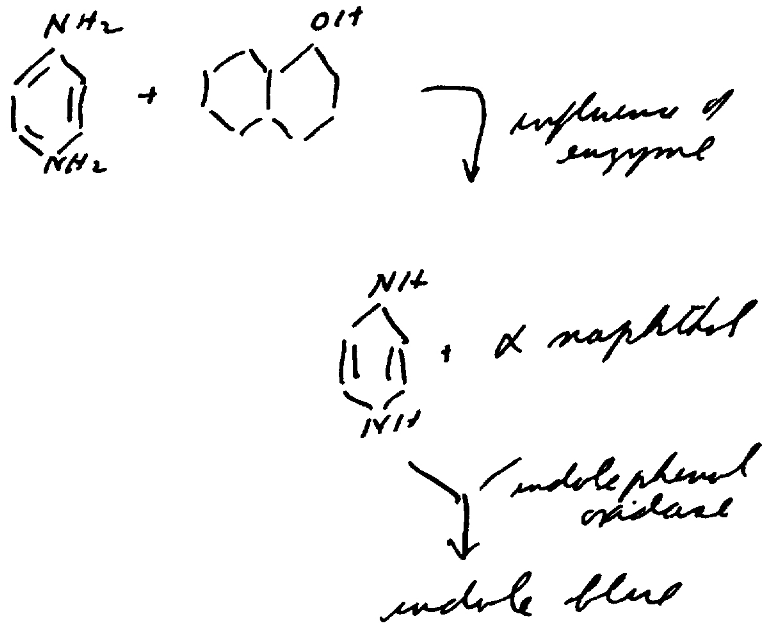
Co I (di P) + Prot A = EtOH dehydrogenase
B = lactic a. "
C = Phosphoglyceraldehyde "

The protein has effect on specificity of reaction

(Warburg)
Hex 6 P - dehydrogen.
Co II (tri P nucleotide) + Prot A, = [illegible]P
" + B, = Glucose "

Aerobic oxidases :- found in all tissues (almost)

- tested for by Nadi reagent



<u>Warburg 1920</u> - aerobic respiration

- narcotics inhibit respirations
- H_2S + HCN " " very effectively

Fe essential in aerobic oxidations

H_2S + HCN reacted specifically with Fe to inhibit aerobic oxidations

- CO poisons aerobic respiration

Fe^{+++} + HCN, H_2S irreversibly

+CO → reversible in presence of lights of certain wave lengths

~~Present views~~: -

1886 - - myohematin isolated from blood
- characteristic absorption band

1925 - renamed cytochrome - found universally in animal tissues
- characteristic abs. bands only in reduced state

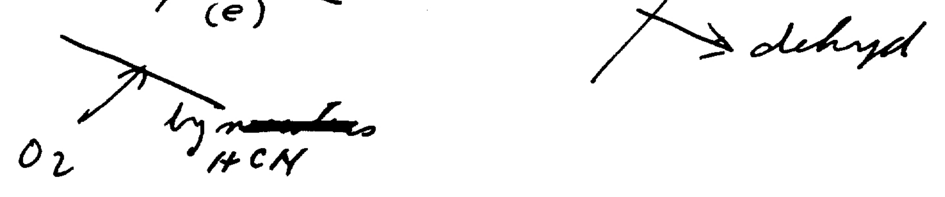
<u>3 cytochromes</u> - A, B + C.
- all have hemin nucleus

- Keilin found narcotics inhibit reduc. of cytochrome
  - urethane etc

$$\begin{tikzcd} Cyt^{Fe++}_{(e)} \arrow[r, shift left, "e"] & Cyt^{Fe+++} \arrow[l, shift left] \\ O_2 \arrow[u] \end{tikzcd}$$

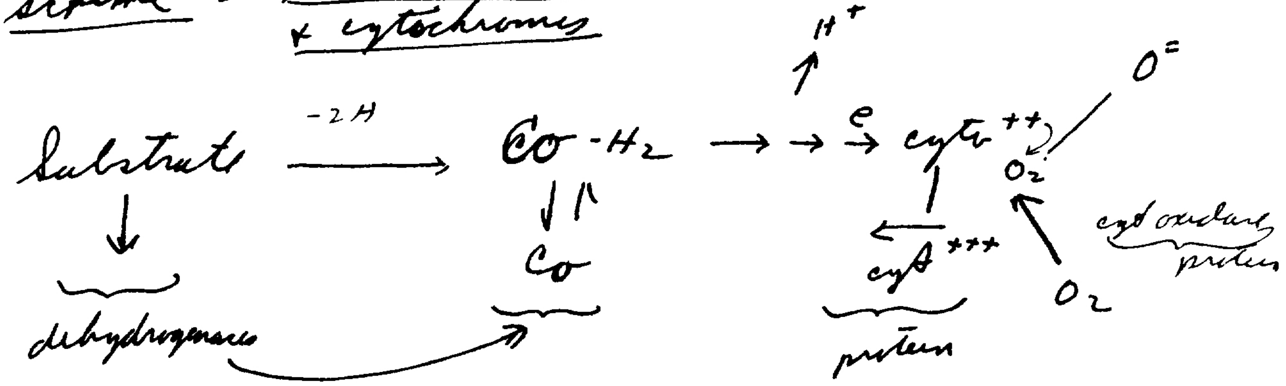
$$O_2 \rightarrow O^{=} (H^+ \rightarrow H_2O)$$

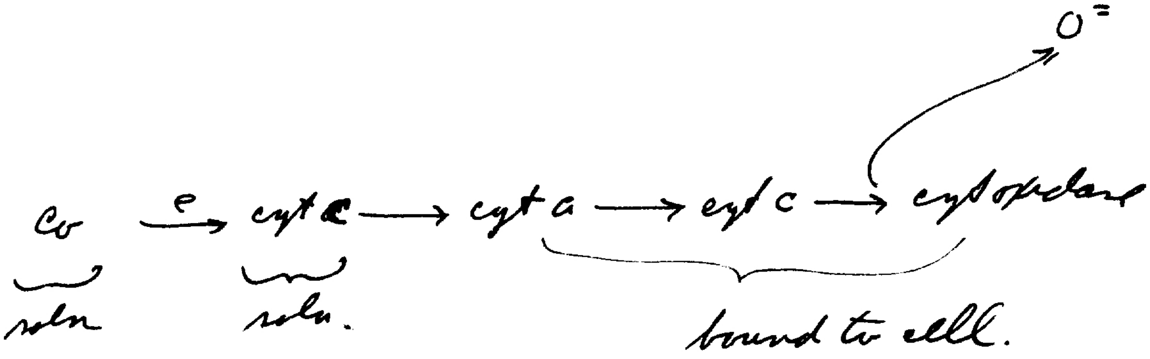
so: -



The enzyme is - indophenol oxidase
- Warburgs resp. enzyme.

Scheme - Relation between dehydrogenating systems + cytochromes

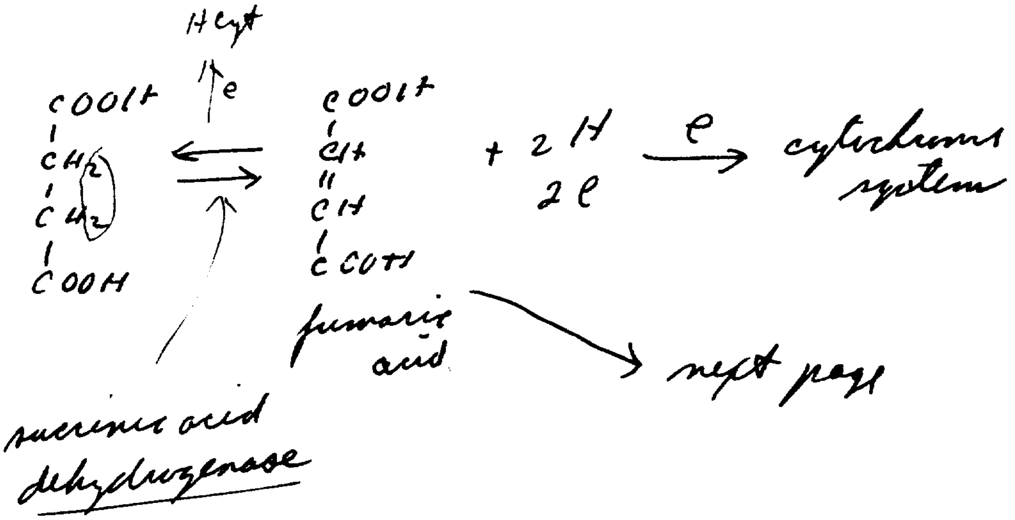


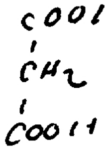


* * *

Szent Györgyi -

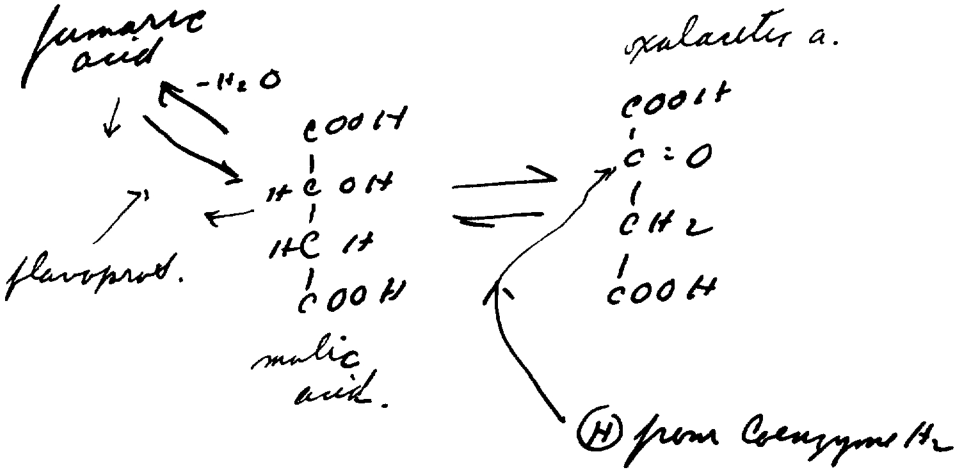
Succinic acid dehydrogenase found to be the only one able to reduce cyt c.

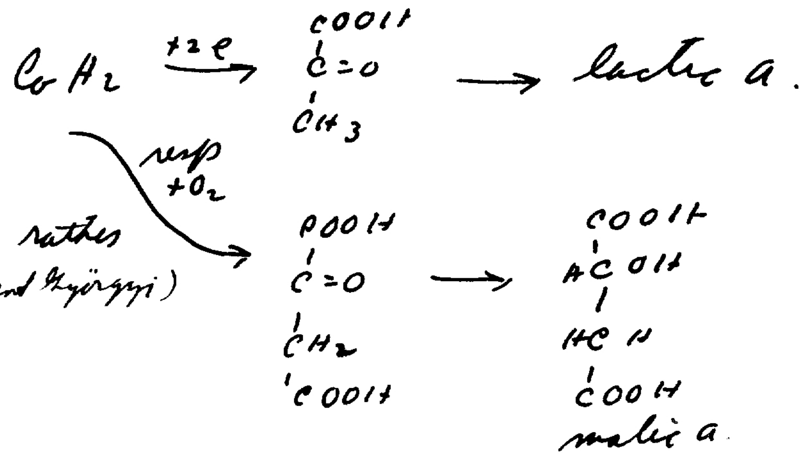




$$\begin{array}{c}COOH\\ |\\ CH_2\\ |\\ COOH\end{array}$$

blocks mech because malonic acid can't be dehydrogenated





1932 - <u>Warburg</u> - isolated <u>iron free</u> respiratory enzyme

- alloxazine

Prot + Co II (pyrid nucleotide)
+ Hex 6P
+ flavine

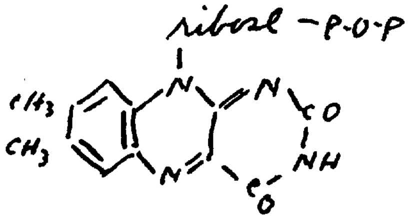
Hex P → Hexonic
Co → Co H₂

$Co H_2 \rightarrow Co + H_2O_2$

↑

flavin
+O₂

1/30/41) Sz. Györgyi - Oxid., Ferm., etc.

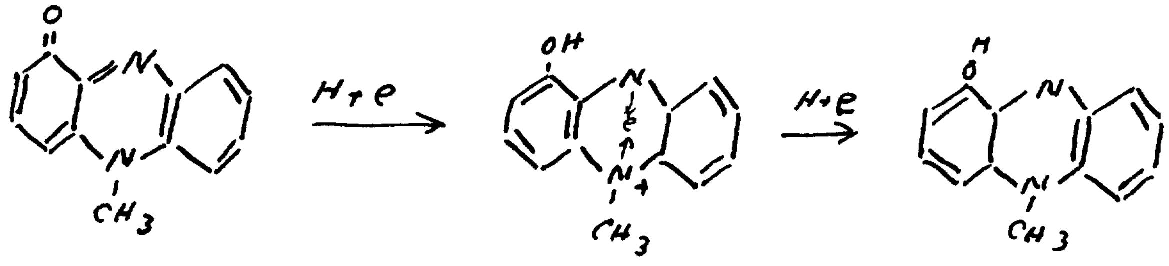


+ specific protein

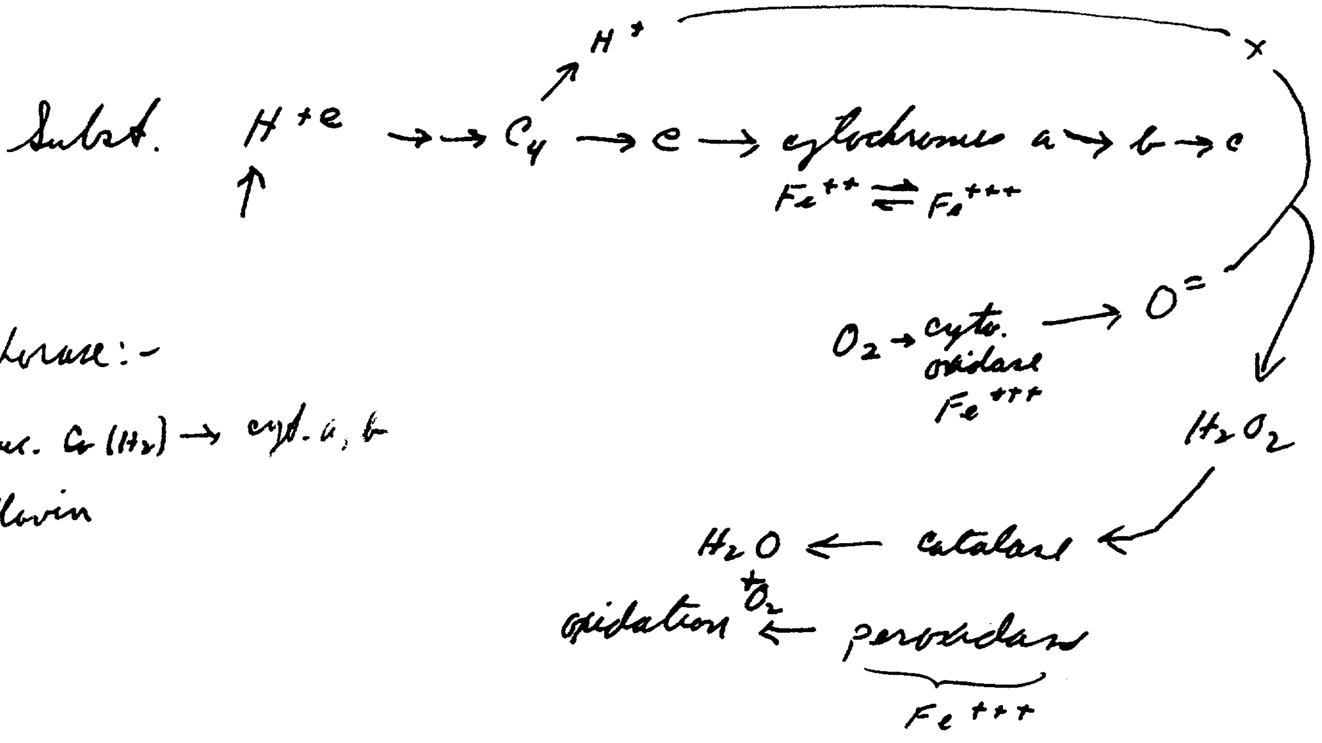
Warburgs yellow enzyme

flavin nucleus

?/ flavin - ribose - P-OP - ribose - adenine
(works on d- amino a.)



pyocyanine



Diaphorase:-

reduc. Co (H2) → cyt. a, b

= flavin

$$\begin{tikzcd}
\text{Succinic} \arrow[d, shift right] & {} \arrow[r, "e"] & \text{cyto} \\
\text{fumaric} \arrow[u, shift right] \arrow[d, shift right, "H_2O \pm"] & & \\
\text{malic} \arrow[u, shift right] \arrow[d, shift right] & \times\ \text{flavoprotein} \arrow[ul, bend right] & \\
\text{oxalic acetic} \arrow[u, shift right] \arrow[ur, bend left] & &
\end{tikzcd}$$

$$\begin{tikzcd}
\text{Hex } P \arrow[r, "-H_2"'] & \text{hexonic acid } P \arrow[dl, bend left] & \\
\text{Prot.} & & \\
\text{Co enz } \underline{II} \arrow[r, shift left] \arrow[d, "\text{flavin}"'] & \underline{\text{Co } H_2} \arrow[l, shift left] & + H_2O_2 \\
O_2 \arrow[ur, bend right] & &
\end{tikzcd}$$

alloxazine $+\overset{\text{prot.}}{A}$ = dihydro-Co $\underline{II} \rightarrow O_2$

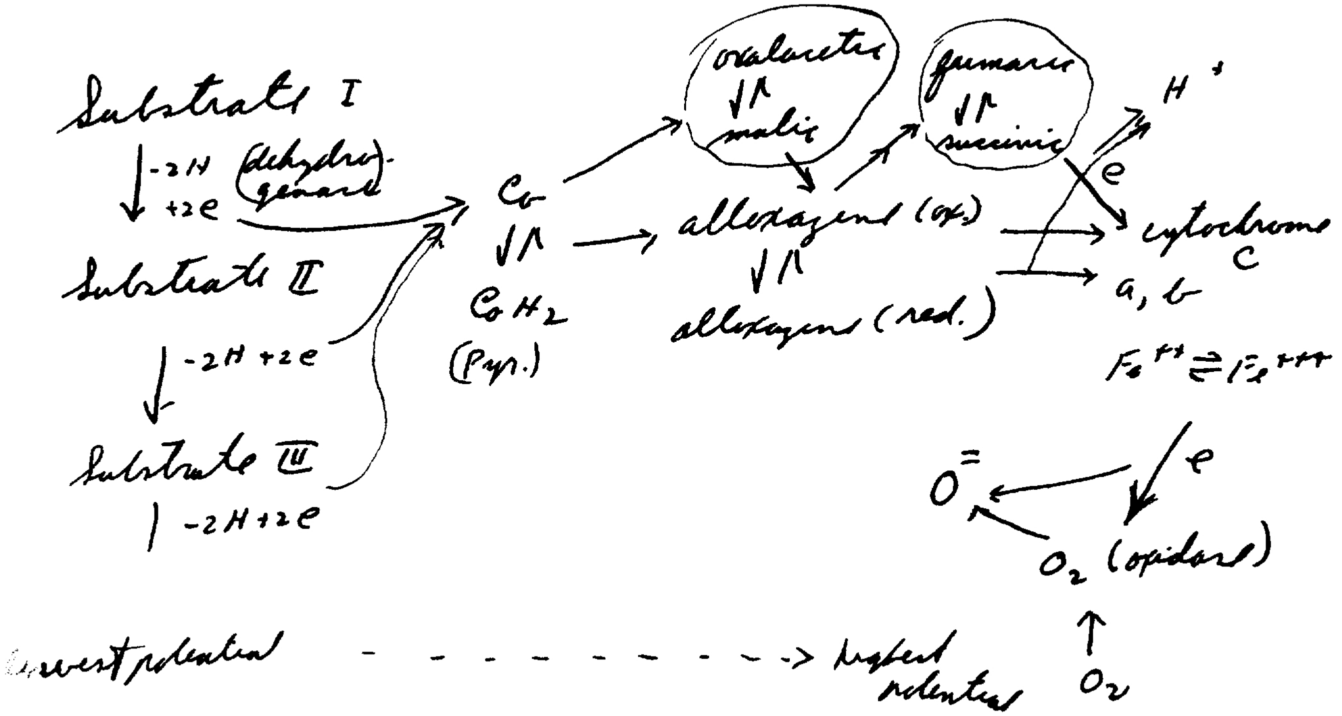
" + adenine nucleotide + prot. B = C_0H_2 I $\rightarrow$ O

" " + prot C = $\rightarrow$ M.B.

" .. + prot. D = d-amino acid oxid.

" " + prot e. = xanthine oxidase

Systems involving diff. cycles + reactions:-



Why do above reactions occur?

- oxidation - red. potential.
- is measure of tendency of system to give up electrons

Classification into 3 groups:-

(1) **Electromotive active** (e → metal (ox))

Fe — cyt, cyt oxid -

Flavoproteins

pyocyanine

(2) **Sluggish systems** (do not react directly with metal)

— slowly to equilibrium

- intervention of dye or one of above electromotive active system

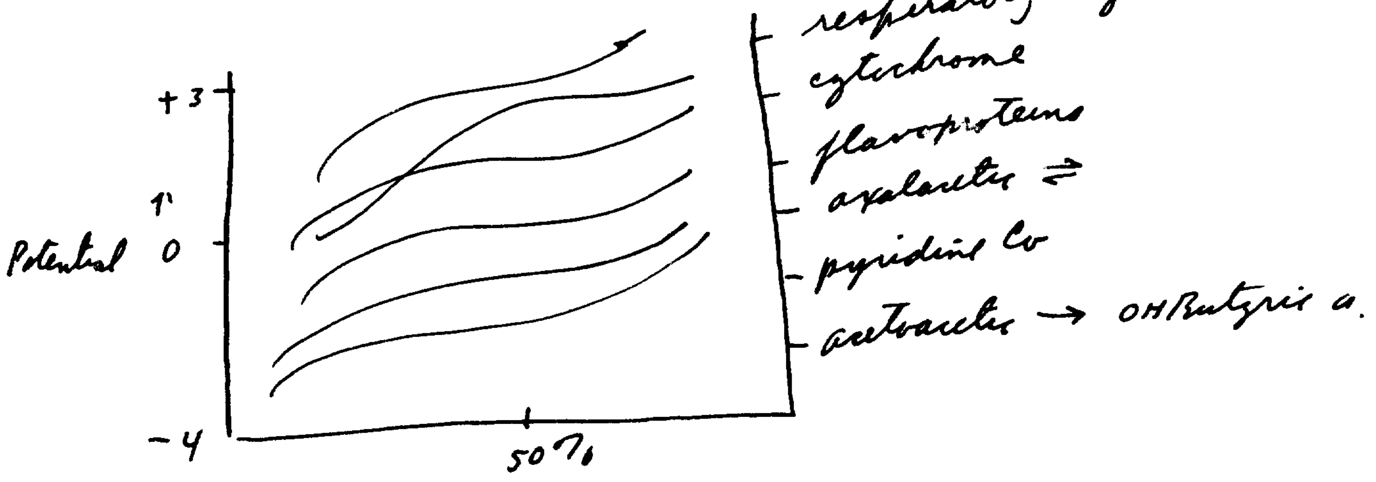
such as:-
- SH
ascorbic (vit c)
pyridine nucl.
thiamin - B_1

(3) Sluggish enzymatic - react slowly

pyruvic ⇌ lactic
acetoacetic → OH butyric
aldehyde ⇌ Et OH

fumaric ⇌ succinic

Illustration :-



Eh at pH 7.0

pyruvic → lactic
methylene violet
formic $\xrightarrow{2H\ 2e}$ $CO_2 + H_2O$

$E_0' = -0.180$
$E_0' = -0.260$
$E_0' = -0.410$

Cbhy $\longrightarrow$ 2H+2e $\longrightarrow$ Co $-$ flavin $+\textcircled{2e}$ $\longrightarrow$ $\textcircled{1e}$ $\begin{array}{c} Cyt^{++} \\ \downarrow\uparrow \\ Cyt^{+++} \end{array}$

Analogy in :—

$Ce^{+++} \rightarrow Ce^{++}$ 1e
$Tl^{+++} \rightarrow Tl^{+}$ 2e } will not react in soln

but $Mn^{++++} \rightarrow \underset{2e}{Mn^{++}}$ or $\underset{1e}{Mn^{+++}}$

∴ Mn act as cat. or intermediate carrier in reaction between Ce + Tl

---

2/4/40

Baldwin pp 73-96
Biolog. Oxidations
Ann Rev. Biochem. '40 p662

	Hemocyanin	Hemerythrin	Chlorocruorin	Erythrocruorin	Hb.
color —	blue	red	green	red	
metal —	Cu	Fe	Fe	Fe	
prosth. gp. —	S-pept.	haem ?	haem	haem	
mol O_2/metal —	1:2	1:3	1:1	1:1	
cc O_2/100cc —	2, 8, 3	2	9	1.5-6.5	9-12
occurrence —	crustacea mollusca	annelids	annelids	invert	vert.

Different Respiratory Mediators in different Organisms

Pyr. Nucleotides coenzymes } H + electron carriers
Flav~~oproteins~~ins }
Thiamins

C_4 di carboxylic acids - H + electron carriers

Substrate →
dehydrogenase

Also depends upon potential of systems
Protein attached to prosthetic groups greatly increase potential of systems.

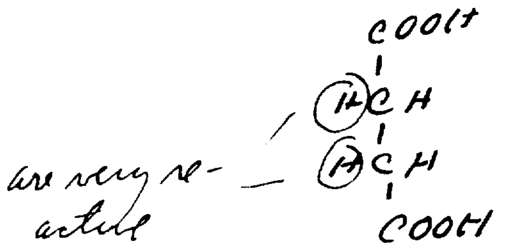
Valence change - gradient change between oxidized & reduced forms

Significance of Structure of Substrates + Mediators

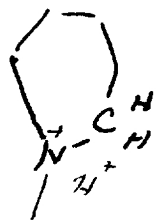
Substrates:

$-C=C-$ can take up + lose electrons readily

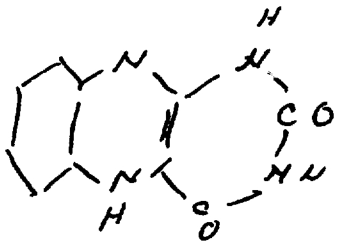
$-C=O$



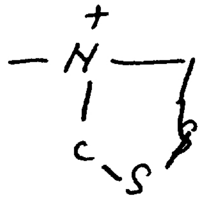
Pyridine nucleotides



Flavins



Thiamines



Cytochromes

Fe^{++} Fe^{+++}

Other mediators

Cu^{+} Cu^{++}

Optional routes in mechanisms of respiration

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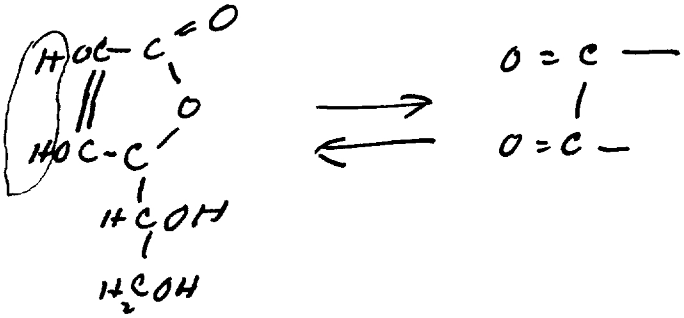
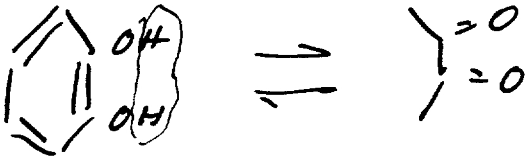
offshoot mechanisms: - In plants

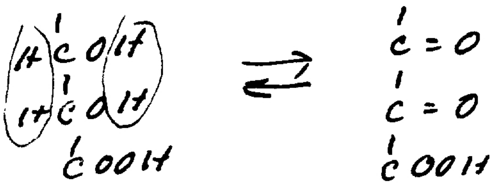
Ascorbic acid — oxidase system (Cu protein) (functions in 1/10 plants)
This may replace cytochrome - cyt oxidase system



catechol - oxidase system (functions in 1/2 of plants)



dioxy maleic acid - oxidase system (functions in all plants)



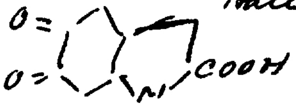
In general - 2 types of respiratory mediators

(1) - production of || bonds
(2) - Metal containing (Fe^{++} - Fe^{+++} etc)

Organic comp'd mediators

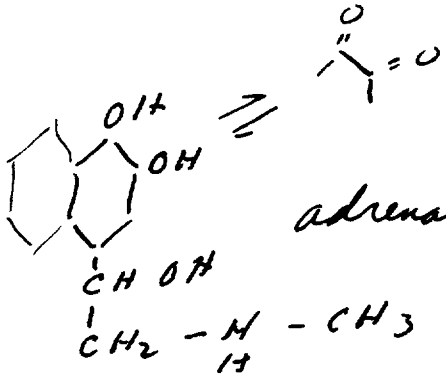
pyrimidines } in both plants & animals
thiamin
flavins

- quinone oxidase systems - catechol +
(in plants + animals) also



Hallochrome

in certain worms

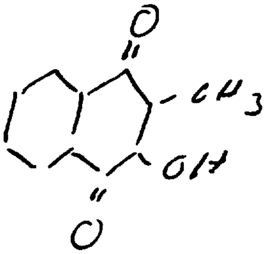


adrenalin

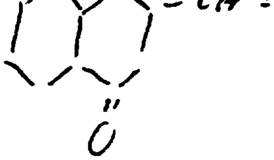
in animals (mammals)

- naphthoquinone

phthiocol
T.B.

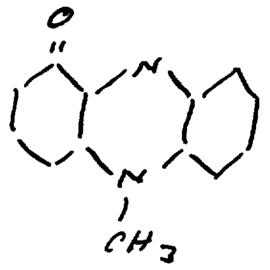


lamatol

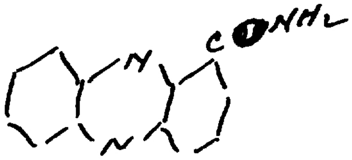


side chain

- phenazines



pyocyanines - bacterial pigment functions in oxidation - reduction



chlororaphine - in bacteria

- ascorbic acid - oxidase

- C_4 dicarboxylic acids -

- glutathione

$R-S\boxed{H \quad H}S-R$ reduced form.

$\downarrow\uparrow$

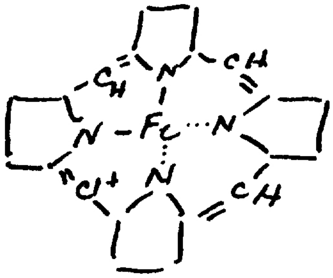
$R-S-S-R$ oxidized form

Metal contg compd mediators

Cu + Fe + Al
Mg - phosphorylations etc.
Vanadium
Zn
Mn

These metals act as catalyzers + are ~~often~~ found in certain enzymes + enzyme systems

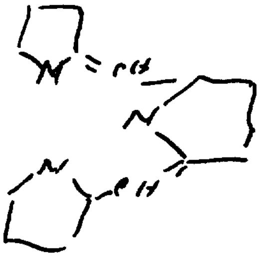
Fe :-



porphin + side chains = porphyrin nucleus

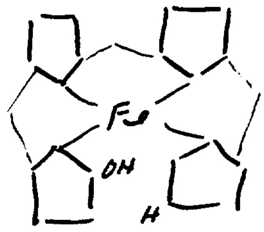
porphyren nucleus + Fe = haemin

prodigiosin - just 3 pyrrole rings - no metal
oxidase functions in certain ~~insects~~ bacteria



Bile pigments

Bile pigment haemochromogens



"cytochrome" acetobacter

chlorophyll

porphyrin nucleus + Mg + phytol side chain

<u>Correlation between various systems</u> : - contg Fe

A Prot. cytochrome - a, b, c.
B cytr - oxidase (O_2)
C catalase
D peroxidase
E (Mg) chlorophyll (Mg)

2/6/41

Biol. Oxid. 254-257

Schmidt:- Amino Acids. Ch V

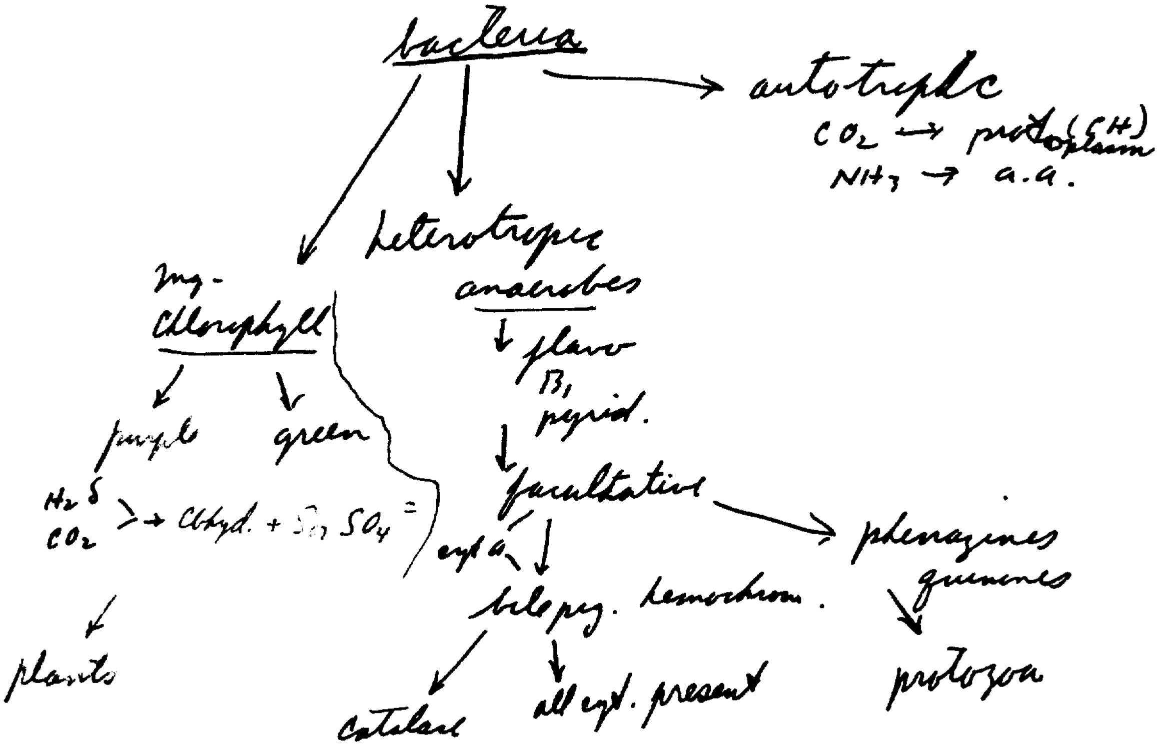
Stephenson Ch. V

Ann. Rev. Biochem. '36 p247

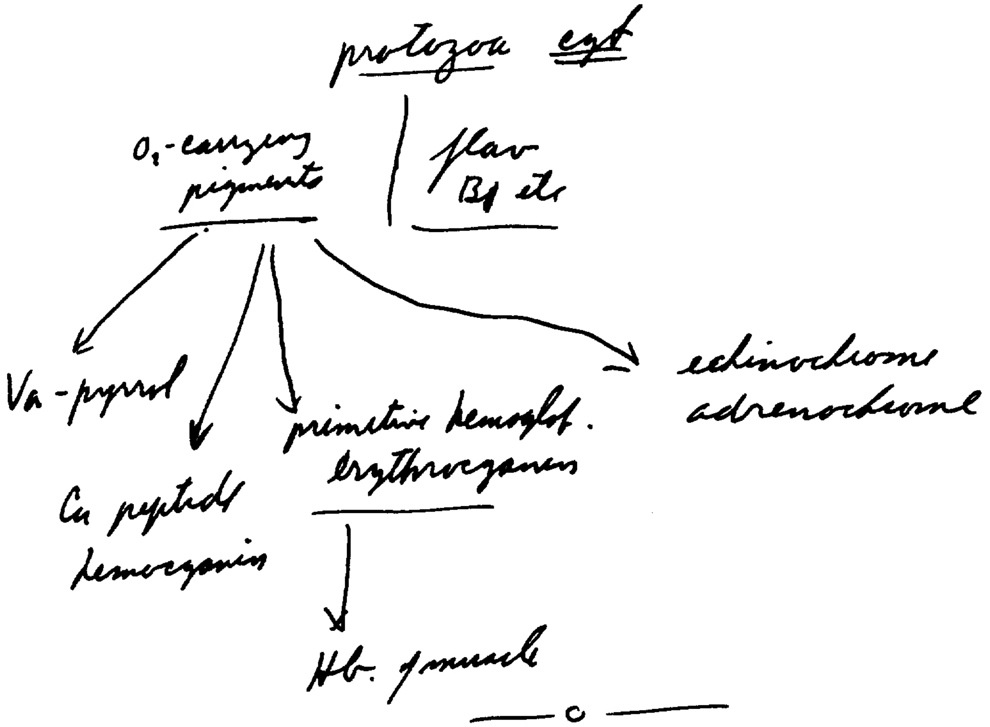
" " " '40 p239

More on Evolutionary Aspects of Past material

- All organisms make use of fundamental oxidative mechanisms including flavins, pyrm. nucleotides, thiamins etc. (Except autotropic bacteria)



Animals



Oxidation of Fats + Amino acids

- Protein → deamination + oxidation
- Deamination in liver + kidney (animals)

Energy from a.a.

- protein sparing action of chhyd. (in animals)

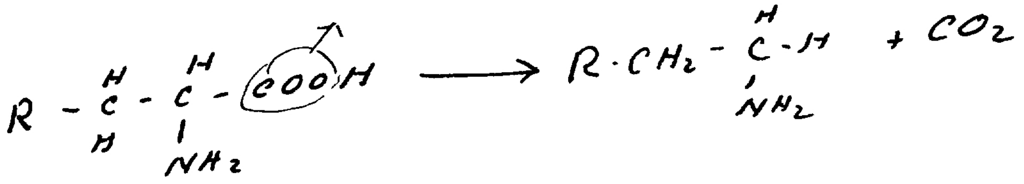
Fats + fatty acids - dehydrogenation + oxidation

- [illegible].

[illegible]

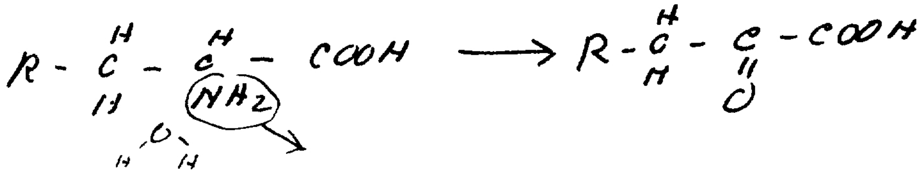
Mechanism of decarboxylation + deamination of amino acids

In bacteria :-



$$R-\underset{H}{\overset{H}{C}}-\underset{NH_2}{\overset{H}{C}}-\boxed{COO}H \longrightarrow R\cdot CH_2-\underset{NH_2}{\overset{H}{C}}-H + CO_2$$

B. Coli - enzymes of a.a. decarboxylation
- these enzymes are specific for different amino acids
- each such enzyme has optimum pH.



$$R-\underset{H}{\overset{H}{C}}-\underset{\boxed{NH_2}}{\overset{H}{C}}-COOH \longrightarrow R-\underset{H}{\overset{H}{C}}-\underset{O}{\overset{}{\underset{\|}{C}}}-COOH$$

$$\overset{O}{H\quad H}$$

B. Coli - enzymes of a.a. deamination
- the deaminase linked with O_2.
- also flavin protein specificity of deaminases

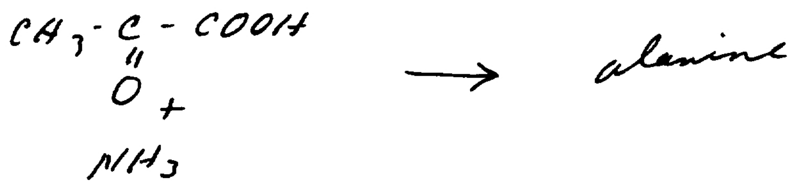
B. Coli - in acid reactions, the decarboxylase is active + deaminase inactive. Vice versa in alkaline reaction.

—— o ——

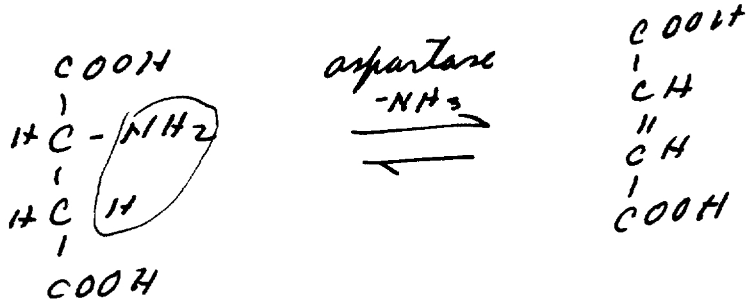
[illegible] :-

Neubauer? :-

$$\text{alanine} \xrightarrow{7} \text{pyruvic} + NH_3$$
$$\text{alanine} \longleftarrow \text{pyruvic} + \text{excess } NH_3$$



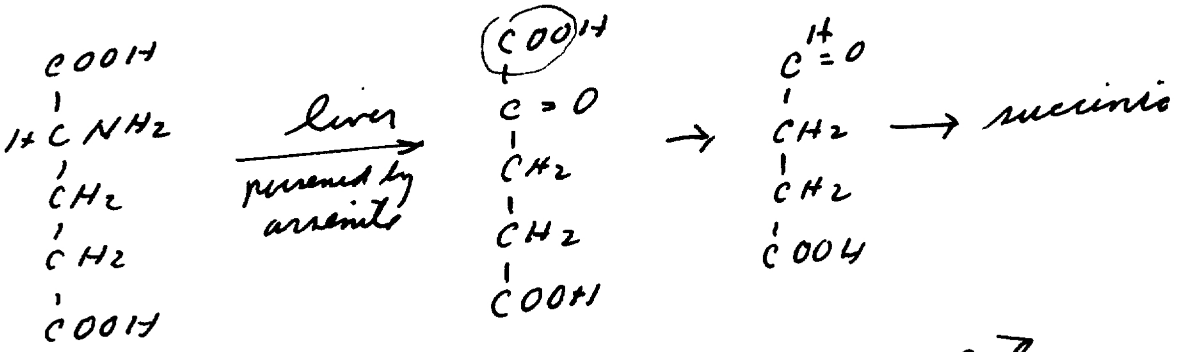
$$CH_3-\underset{\underset{O}{\|}}{C}-COOH + NH_3 \longrightarrow \text{alanine}$$



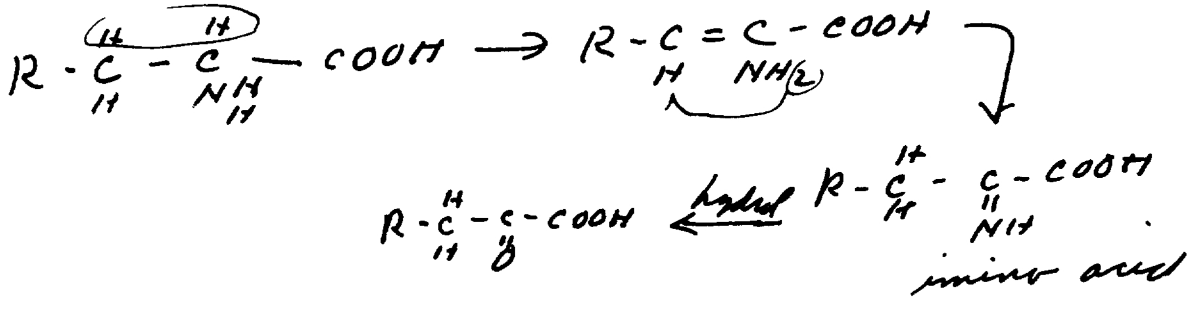
Aspartase of B. Coli requires a coenzyme, namely as:-
adenine phosphate flavoprotein

— o —

In animals:- deamination

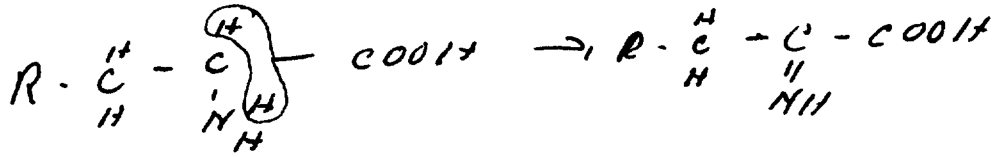


Deamination in liver without decarboxylation

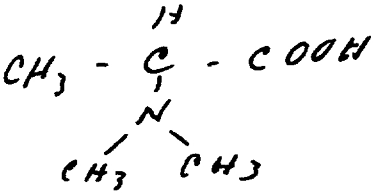


$$R-\overset{H}{\underset{H}{C}}-\overset{H}{\underset{NH_2}{C}}-COOH \longrightarrow R-\underset{H}{C}=\underset{NH_2}{C}-COOH \longrightarrow R-\overset{H}{\underset{H}{C}}-\underset{\underset{NH}{\|}}{C}-COOH \xrightarrow{hydrol} R-\overset{H}{\underset{H}{C}}-\underset{\underset{O}{\|}}{C}-COOH$$

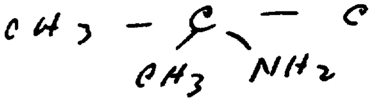
more probable direct imino formation



$$R-\underset{H}{\overset{H}{C}}-\underset{N\,H\,H}{\overset{H}{C}}-COOH \rightarrow R-\underset{H}{\overset{H}{C}}-\underset{NH}{\overset{}{C}}-COOH$$

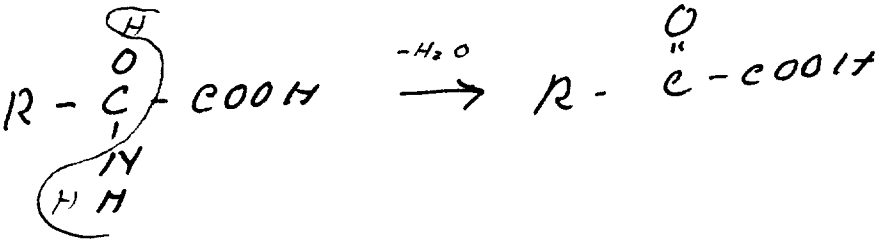


$$CH_3-\underset{N(CH_3)(CH_3)}{\overset{H}{C}}-COOH \rightarrow \text{not deaminated}$$



$$CH_3-\underset{CH_3\ NH_2}{C}-COOH - \text{not} \ "$$

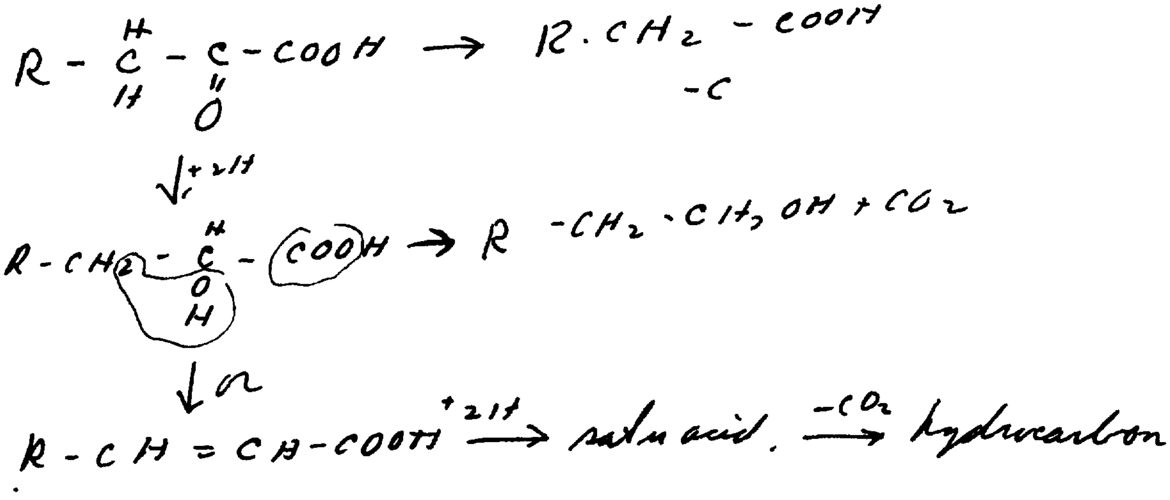
∴ a H must be on α C & on N atoms for deamination



$$R-\underset{N\,H\,H}{\overset{H\,O}{C}}-COOH \xrightarrow{-H_2O} R-\overset{O}{\overset{\parallel}{C}}-COOH$$

—— o ——

<u>Decarboxylation of α-keto acids from a.a. deamination</u>



$$R-\underset{H}{\overset{H}{C}}-\underset{O}{\overset{}{C}}-COOH \rightarrow R\cdot CH_2 \begin{matrix}-COOH\\ -C\end{matrix}$$

$$\downarrow +2H$$

$$R-CH_2-\underset{OH}{\overset{H}{C}}-COOH \rightarrow R-CH_2\cdot CH_2OH + CO_2$$

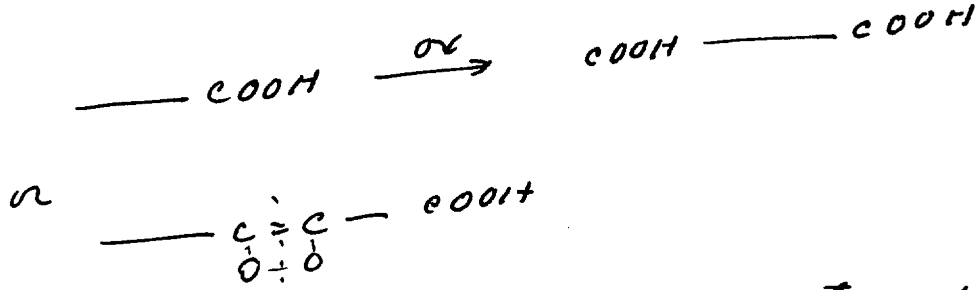
$$\downarrow \text{or}$$

$$R-CH=CH-COOH \xrightarrow{+2H} \text{satu acid.} \xrightarrow{-CO_2} \text{hydrocarbon}$$

Many of the coenzyme involved in a.a. oxidative mechanisms are the same or similar to those in oxidative mechanisms of cbhyd.

Oxidation of Fats :-

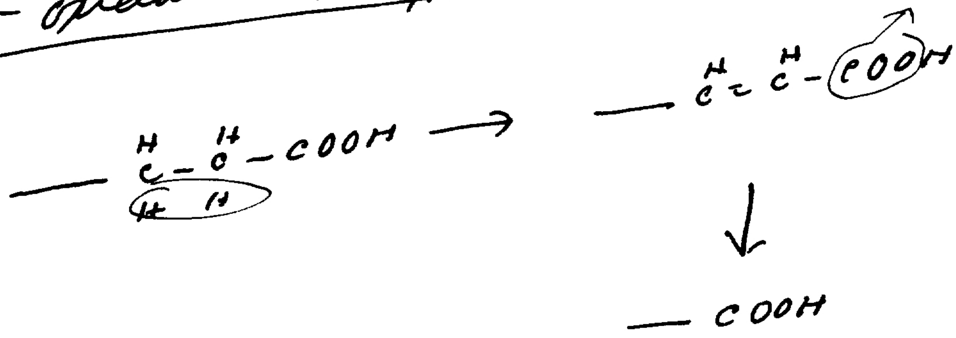
. involves typical dehydrogenases.



$-COOH \xrightarrow{or} COOH-COOH$

or $-\underset{O}{C}=\underset{O}{C}-COOH$

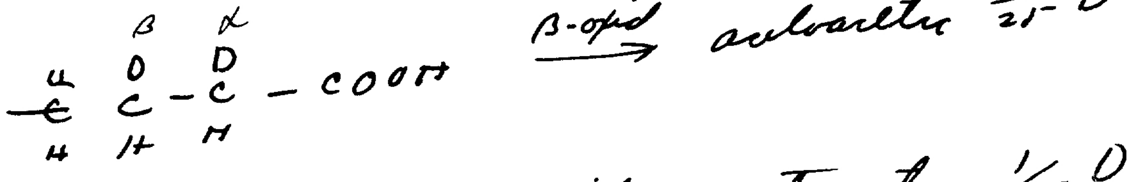
β-oxidation is typical oxidation of fats :.



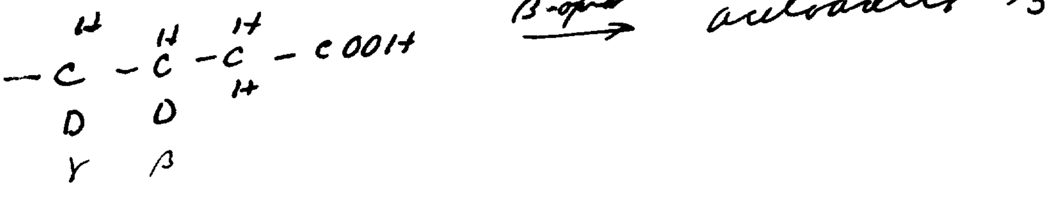
$-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-COOH \rightarrow -\overset{H}{C}=\overset{H}{C}-COOH$

$\downarrow$

$-COOH$



$-\underset{H}{\overset{H}{C}}\;\underset{H}{\overset{\overset{\beta}{D}}{C}}-\underset{H}{\overset{\overset{\alpha}{D}}{C}}-COOH \xrightarrow{\beta\text{-oxid}}$ acetoacetic $\frac{1}{21}$- D



$-\underset{\underset{\gamma}{D}}{\overset{H}{C}}-\underset{\underset{\beta}{D}}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-COOH \xrightarrow{\beta\text{-oxid}}$ acetoacetic 1/5- D

2/11/41

Clifton + Logan (1939)
J. Bact. 37, 523

Winzler + Baumberger ('38)
J. Cell. Comp. physiol.

Ann. Rev. Biochem '38-40 Carbohyd. Met.

Fat + Carbohydrate Syntheses in Animal Organisms

Utilization of Energy from oxidation :-

- Syntheses
- Growth etc.

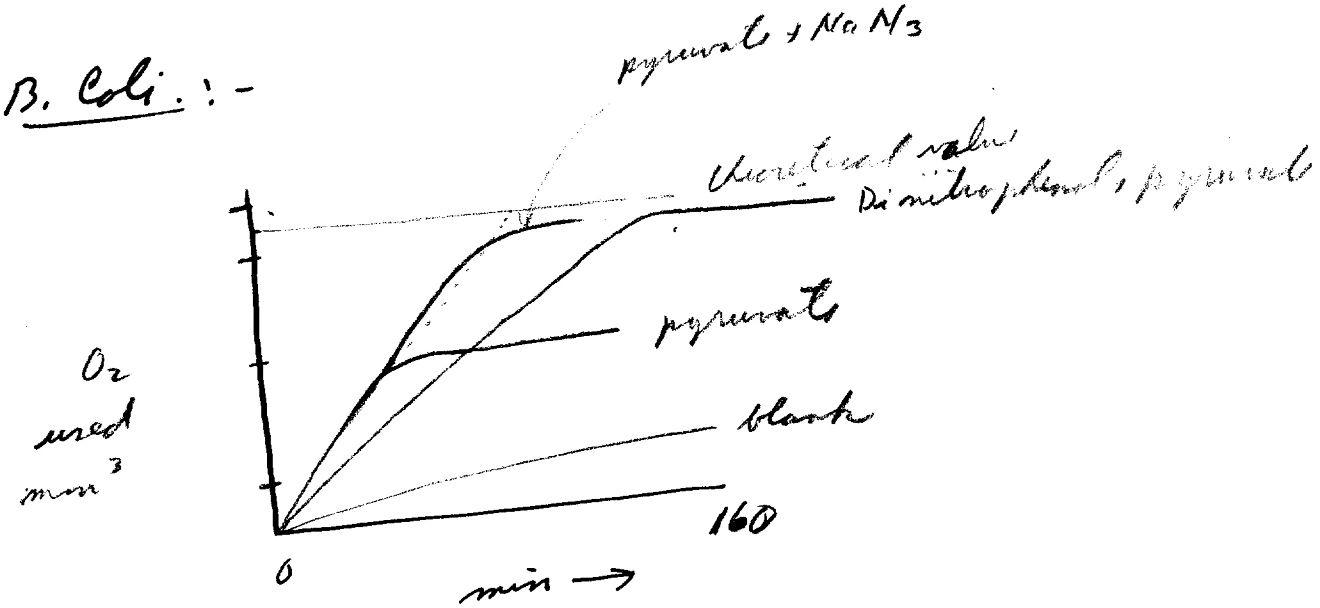
Deficiency of aerobic verses anaerobic oxidation :-

- greater am't of energy available under aerobic oxidation
- cited examples of bacteria + yeast + molds production of or rather synthesis of carbohydrates + fats under aerobic + anaerobic conditions
- am't + kind of substrates present also have control on rate of syntheses of fats + carbohydrates.

Rate of respiration & syntheses compared :-

- Respiration is incomplete since less than theoretical am't of O_2 per unit wt. substrate is used.
- Respiratory quotient is near 1 in case of organisms metabolizing cbhyds.

B. Coli :-



In the case of the poisons NaN_3 & DNP, the oxidation of pyruvate is almost complete because poisons inhibit synthetic processes.

- 2 pyruvate + $3O_2 \longrightarrow 2(CH_2O) + 4CO_2$

1/3 substrate is synthesized to cbhyd.

glucose is almost 50% assimilated

Synthetic processes may be poisoned selectively

<u>The ratio of am't oxidized + synthesized is constant in spite of changes in conc. of substrate.</u>

—— o

$$\begin{tikzcd}
\text{pyruvate} \arrow[r, "\text{red}", "H_2\text{ dehydrogenase}"'] & \text{lactic acid} \\
\text{formate} \arrow[u, bend left] \arrow[r, "\text{oxid}", "\text{dehydrogenase}"'] & CO_2 + H_2O
\end{tikzcd}$$

<u>toluene</u> does not affect dehydrogenases but affects coupling link between oxidation + syntheses, ∴ inhibits syntheses.

Efficiency of aerobic verses anaerobic synthesis:-

	ΔF synth	−ΔF dissimilation	% assimilated	% loss	efficiency %
glucose oxid	7,320	700,000 100x	73%	26%	2,88
" ferm.	7,320	66,000 9x	24%	70%	4.62
acetate oxid	39,850	230,000 6x	41%	58%	12,2

as these two values approach each other the synthesis mechanism approaches 100% efficiency animals :-

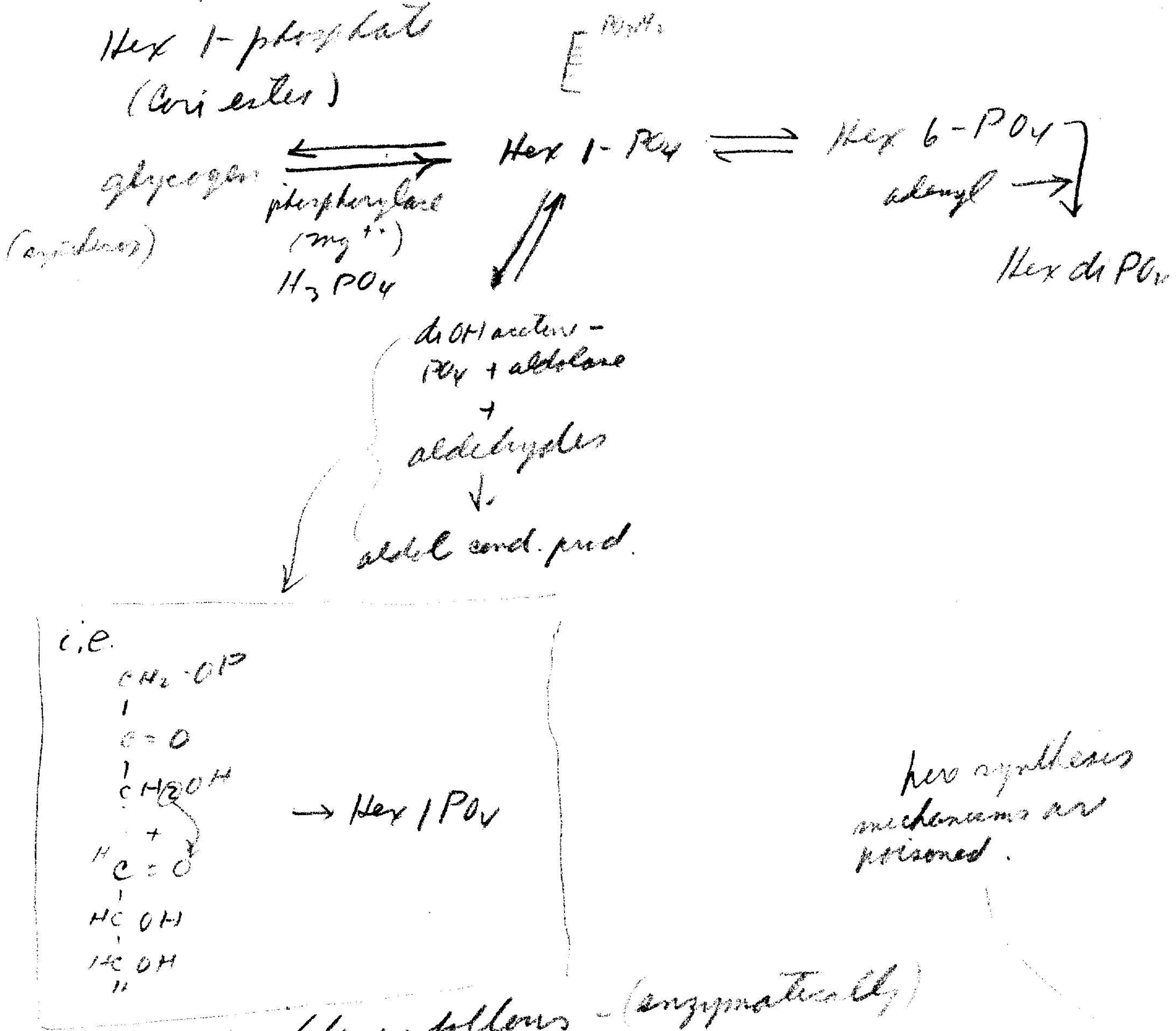
Substrate + O_2 energy $\longrightarrow$ $(CH_2O)_n + CO_2 + H_2O$

green plants :-

$CO_2 + H_2O \xrightarrow[\text{energy}]{\text{radiant}} (CH_2O)_2 + O_2$

— o —

Mechanisms of Carbhyd. Synthesis in Animals :-



all are reversible as follows - (enzymatically)

$Hex P \rightleftharpoons$ triose $PO_4 \rightleftharpoons$ glyceric a. $\rightleftharpoons$ P-pyruvic

$\downarrow$ energy coupled (not re-versible)

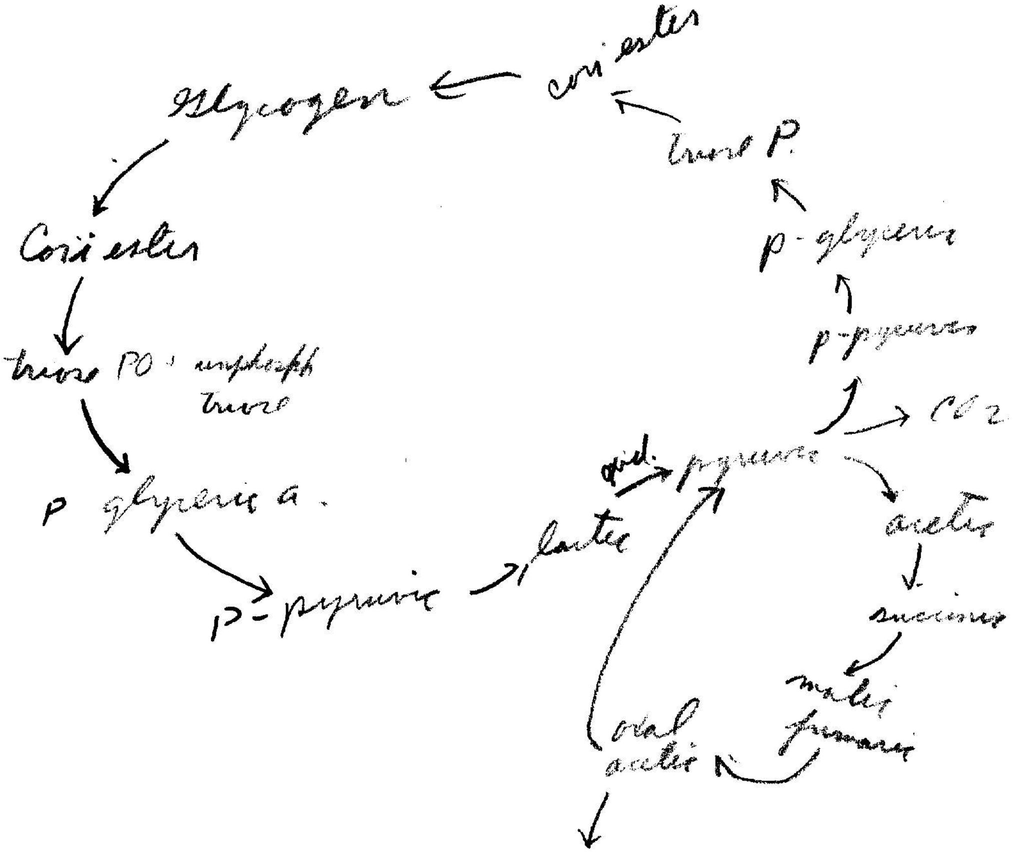
$\Longleftarrow$ pyruvic

Linking of energy with syntheses —

$$ATP + CoH_2 + 2\,P\text{-glyceric a.} \rightleftharpoons 2\,\text{triose}\,PO_4 + 2\,Co + H_3PO_4 + \text{adenylic a.}$$

this energy could be used

In animals, syntheses is related with this dissimilation: —



$$\text{Cori ester} \xrightarrow[\text{yeast phosphorylase}]{\text{liver or}} \text{glycogen}$$

$$\text{"} \xrightarrow[\text{phosphorylase}]{\text{potato}} \text{starch}$$

Specificity of phosphorylase protein decides whether starch, glycogen etc shall be synthesized.

Synthesis is the reversal of dissimilation, in case of carbohydrate synthesis.

* * *

Baldwin · Ch IV + V

Ann. Rev. Bioch. '40 p 282-284

Interrelations · a. acids:.

Krebs - *Enzymologia I*, 53 ('39)

What happens to N of a. acids + purines when metabolized in animals:-

- simplest form of excretion is NH_3 (bacteria etc.)
- tri methylamine oxide in some aquatic forms (non toxic form) That is marine aquatic forms.
- amphibian forms detoxify NH_3 by production of urea
- terrestrial types excrete urea
- birds + reptiles excrete a.a. N as uric acid.

- Supply of water detn's whether urea or uric acid is excreted

Production of urea :- see text.

ornithine + CO_2 + NH_3 → citrulline + NH_3

This scheme involves enzymes.

In mammals - break down of purines :-

purine split to give allantoin (enzymatic action)

Excretion forms of N of a.a. ——— o ——

amino acid NH_2 → NH_3 → $(CH_3)_3N=O$

NH_3 → NH_3

NH_3 → ornithine → urea (arginase)

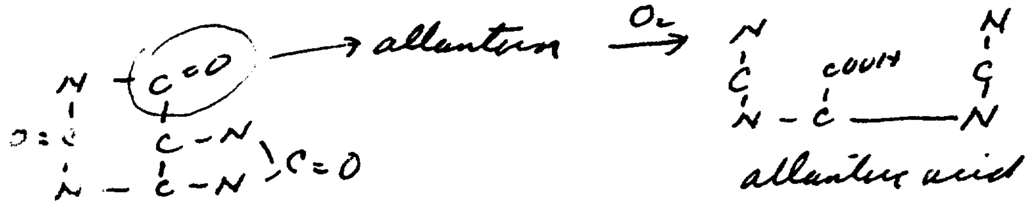
allantoic acid → urea

allantoin → allantoic acid

Excretion forms of N of purines

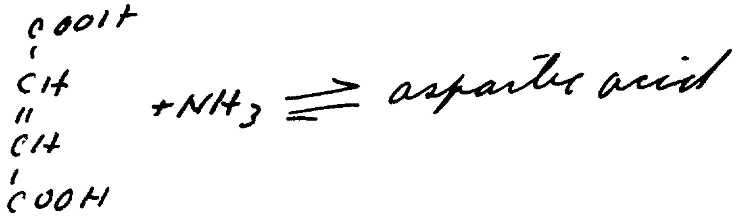
purine N → xanthine hypoxanthine → uric acid

uric acid → allantoin



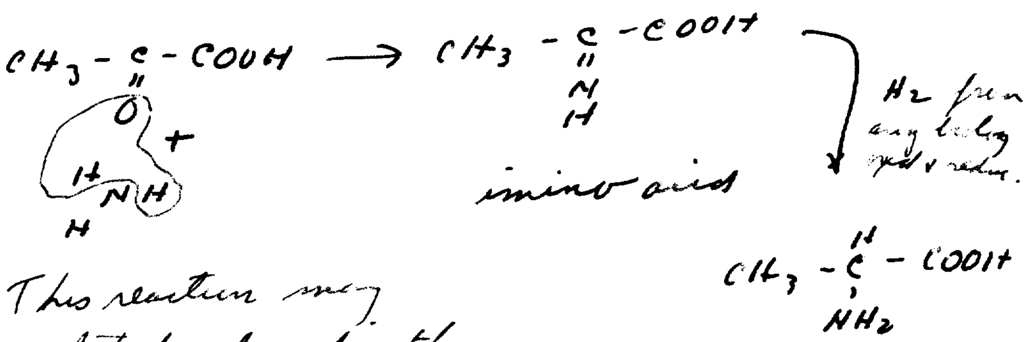
Syntheses ~~from~~ of a. acids :- Three types

(1) Aspartase



$$\begin{matrix} COOH \\ | \\ CH \\ \| \\ CH \\ | \\ COOH \end{matrix} + NH_3 \rightleftharpoons \text{aspartic acid}$$

(2) addition of NH_3 to α keto acid (pyruvate)



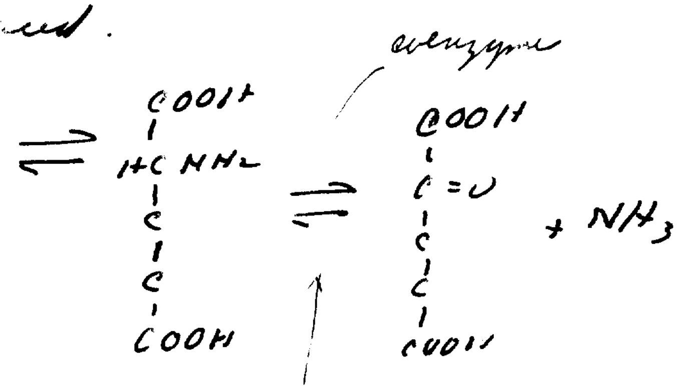
$$CH_3-\underset{\underset{O}{\|}}{C}-COOH + H-NH-H \longrightarrow CH_3-\underset{\underset{H}{\overset{\|}{N}}}{C}-COOH \longrightarrow CH_3-\underset{NH_2}{\overset{H}{C}}-COOH$$

imino acid

H_2 from any body oxid + reduc.

This reaction may not take place directly

(3) Thru dicarboxylic acid.



$$\text{Glutamic acid} \rightleftharpoons \begin{matrix} COOH \\ | \\ HC\,NH_2 \\ | \\ C \\ | \\ C \\ | \\ COOH \end{matrix} \rightleftharpoons \begin{matrix} COOH \\ | \\ C=O \\ | \\ C \\ | \\ C \\ | \\ COOH \end{matrix} + NH_3$$

coenzyme

intermediate imino acid

Glutamic acid + Co $\rightleftharpoons$ imino acid + CoH_2

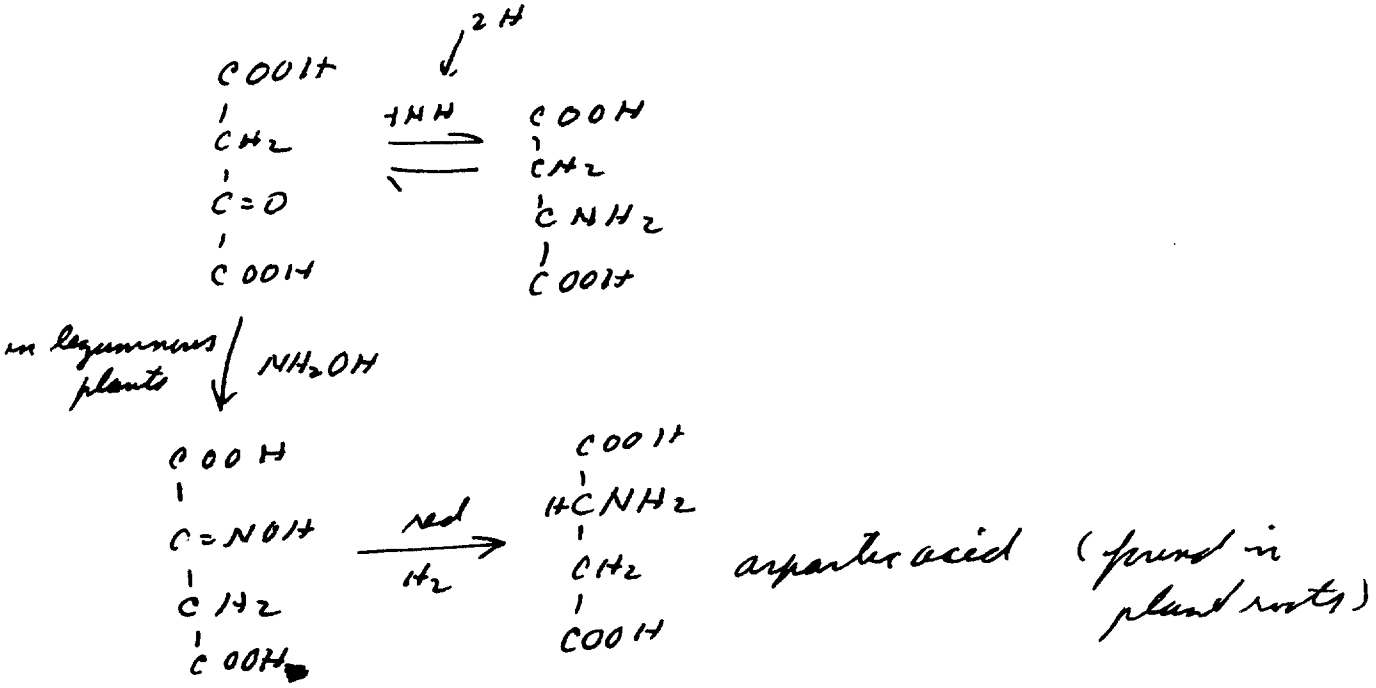
↑ enzyme

↓↑ H_2O

keto acid + NH_3

Co I or Co II

liver — yeast



$$\begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ C=O \\ | \\ COOH \end{array} \underset{}{\overset{\downarrow 2H}{\overset{+NH}{\rightleftharpoons}}} \begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ CNH_2 \\ | \\ COOH \end{array}$$

in leguminous plants $\downarrow NH_2OH$

$$\begin{array}{c} COOH \\ | \\ C=NOH \\ | \\ CH_2 \\ | \\ COOH \end{array} \xrightarrow[H_2]{red} \begin{array}{c} COOH \\ | \\ HCNH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

aspartic acid (found in plant roots)

Ketoglutaric acid & oxalacetic acids only dicarboxylic acids which ~~not~~ can be directly enzymatically converted to a. acids

——— o ———

(4) <u>Another method of a.a. synthesis</u> :- transference of NH₂ groups to α keto dicarboxylic acids

Glutamic a + pyruvic ⇌ keto-glut A + alanine

enzyme (not specific for glutamic or pyruvic)

Glutamic: also aspartic

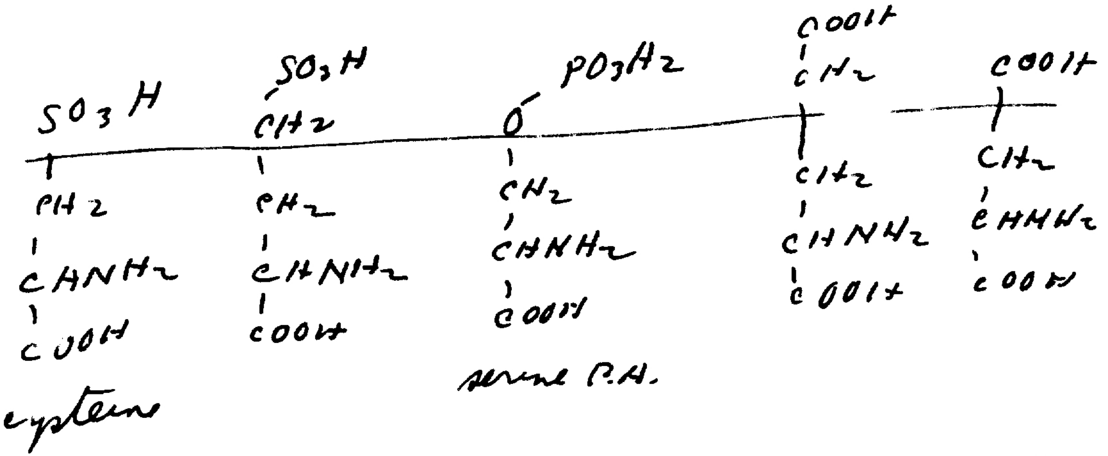
pyruvic: other α keto acids:-
butyric
caproic
oxalacetic

keto-glut A: oxalacetic

alanine: aspartic

pyruvic + NH_2 $\xrightarrow[\text{tissue}]{\text{mus.}}$ alanine + keto acid (di carbox)

However, this amino group may be obtained not only from di -COOH acids but also from acids such as :-



cysteine

serine P.A.

All acids with group as indicated below line with give up $-NH_2$ group to α keto acids such as pyruvic acid.

— o —

this will not occur except thru some decarboxylic acid.

lysine ↗ → pyruvic a ——→ alanine

↓

α keto

lysine → glutaric ↘ pyruvic acid → alanine

↓

α - keto

Synthesis of a.a. + relation to evolution :- see Baldwin

	N need	C - source of energy	
blue-gr. algae	N_2 (atm)	CO_2	light (H_2O)
photosynthetic autotropic bacteria	NH_3	CO_2	light (H_2S)
chemosynthetic	NH_3 or NO_3^-	CO_2	$H_2S \rightarrow S$
facultative heterotropic bacteria	NH_3	CO_2 (CH_2O)	inorg. oxid. oxid of (CH_2O)
obligate heterotropic bacteria	NH_3	(CH_2O)	oxid of (CH_2O)
>N_2 fixation bacteria	N_2 NH_3	"	"

plants — NH_3 or NO_3^- — CO_2 — light

Bact rhizobia — N_2 — (CH_2O) — oxid of (CH_2O)

Dispensibility + Indispensibility of a.acids

Essential a.acids :- see text.

They have either branched chains,
complex rings or imidazol ring
such as histidine

2/18/41)

Lwoff - Ann. Inst. Past
61, 5-80 (1938)

Knight - Bact. Nutrition (1936)
Monograph - pp. 137-161

Williams - Biol. Rev. 16, 49 ('41)

Growth factors in bacterial metabolism :-

- Complex nitrogenous substances are very often growth factors rather than an indispensible source of N for bacterial growth.

Correlation between growth factors, ability to synthesize + position of organism in evolutionary scale.

- As we proceed up evolutionary scale, ability of organism to synthesize growth factors (Vitamins in high evolutionary scale) seems to fall off. These factors must be obtained from outside source.

Growth factors are important & indispensible.

- they sometimes form prosthetic groups of respiratory enzymes.
- factors must be present in almost all forms of life

Growth factors which are constituents of respiratory enzymes

Haematin (Fe containing porphyrin)
Nicotinic acid
Thiamin

As regards bacterial growth

- It is sometimes found that certain strains of bacteria are incapable of synthesizing a few growth factors necessary for normal growth. These factors must be supplied.

Example:-

Hemophilic bacteria.-

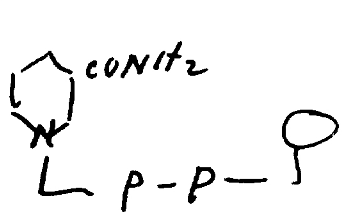
- must obtain haematin for aerobic growth
  - also a pyridine nucleotide.

Staph aureus

- requires Vit B_1 (from outside source) for normal growth.
- nicotinic acid etc.

Development of organisms as regards their synthetic ability

Coenzymes:



Some organism just requires nicotinic acid & can synthesize from there on. Other organisms must be supplied with the whole molecule.

Similarly with Vit. B_1

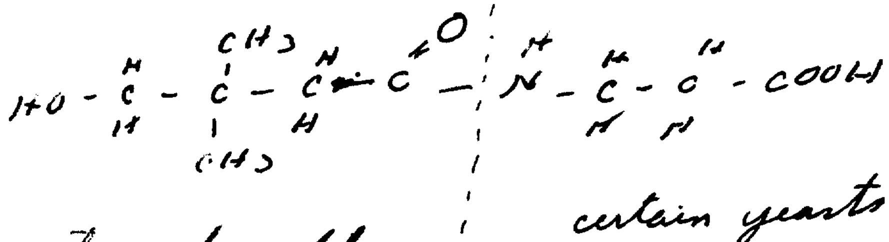
Vit B_1 = pyrimidine + thiazole

Some organisms require only one or the other of above constituents for complete synthesis of B_1. Other organism can't synthesize B_1 at all from one or both of above constituents but must be supplied with B_1.

Growth factors required by many forms of life (must be supplied)

Vitamin B_1 - required by many animals & micro-organisms. Ability to synthesize is lost by these forms of life

Pantothenic acid :- an essential factor for yeasts



$$HO-\underset{H}{\overset{H}{C}}-\underset{CH_3}{\overset{CH_3}{C}}-\underset{H}{\overset{H}{C}}-\overset{O}{C}-\overset{H}{N}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-COOH$$

certain hemophilic bacteria can form pant. a. from above portion of its molecule.

certain yeasts can synthesize pant. a from alanine portion above

Biotin :- required by certain fungi (in their respiratory cycles)

Vit H :- forms dermatitis in rats if not present when rat diet is high in egg white.

Vit. B_6 - bacteria, insects & animals require it

- has some function in plants.

Inositol (cyclohexanol) :- factor in mice.

- prevents falling hair (in mice)

Cholesterol :- required by protozoa etc
- this might be a phylogenetic precursor of Vit D.

Many water soluble B factors :-

Some of the above may be stimulatory growth factors :- that is they stimulate its synthesis which is normally slow in the organisms

Only portions are necessary for complete synthesis of the growth factor in certain other organism

In high forms of life practically none of these growth factors are synthesized. All must be supplied.

Where these growth factors are synthesized, it is accomplished by specific, catalytic proteins, namely enzymes.

2/20/41)

Stephenson ch XI
Knight · Monograph 162 - 175
Perspect. Biochem p91 - 98

Review of Growth Factors & Vitamins

- evolutionary development.
  - loss of ability to synthesize (progressive loss)

- essential factors - sometimes taken care of by symbiotic relationship

As regards plants:-
- certain portions of plant may have lost synthetic ability. But plant as a whole can synthesize all necessary growth factors.

- Generality of essential growth factors (necessary for all forms of life)

- Loss of synthetic ability is attributed to what?
  - due to loss of ability to produce enzyme which is necessary for synthesis.
  - loss or gain of ability to synthesize based upon environmental changes

Comparison of Enzymes in Forms of life along evolutionary scale - Modifications of enzymes due to environment.

- by varying conditions of a specific organisms, certain enzymes of organism can be modified to adapt themselves to environment.

Specific example:- B. Coli. - does not ferment lactose.

if, however, grown on lactose containing medium, it will ferment lactose after it has completely used up other available sugars. These secondary growths of B. Coli can hydrolyze lactose for many generations

This is a sort of mutation + natural selection

- change chemical environment of organism + modifications of certain of its enzymes occur.

Karstrom 1930 - B. aerogenes

Fermentation of xylose

Grow xylose - organism on lactose medium, suspension of these organisms would be xylose negative if suspendended in xylose medium containing no N. If a N source is added, fermentation of xylose would occur.

Point is: that organism did lack enzyme but ~~no~~ was able to synthesize it upon having an ~~avail~~ available N source.

Enzymes which can be lost or gained, depending upon environmental change:— <u>adaptive enzymes</u> as compared with <u>constitutive enzymes</u>.

<u>A. aerogenes</u>

Subst ferm by suspension

grown on:	xylose	arabinose	glucose
arabinose	−	+	+
xylose	+	−	+
	adaptive		constitutive

Production of adaptive enzymes is in response to stimulus in form of substrate upon which it is grown.

Hydrogenlyase - in B. Coli - an adaptive enzyme

formic $\rightarrow CO_2$

$\rightarrow H_2$

However, does not thrive any better on formate containing medium than on a normal medium.

Inhibition of adaptive enzymes

Production of an adaptive enzyme for a specific polysaccharide - Du Bois - not natural selection since organisms used were suspended in thick syrupy poly sacch. soln + no growth occurred but adaptive enzyme for hydrolysis of this polysaccharide was produced.

Yudkin :- mechanism of production of adaptive enzymes

inact. precursor $\rightleftharpoons$ active enzymes

If appropriate substrate is present, reaction or equilibrium goes to right. also By the substrate hydrolyzed or its end products of hydrolysis

Virtanen :- two types of enzymes could be classified by whether they played an essential role in metabolism or whether they prepared substrate for metabolism by organism

adaptive	**constitutive**
preparatory	essential
hydr. starch	trioses
" lactose	glucoses etc
" protease	
etc	

Further classification of enzymes

(1) - enzymes for specific subst + reaction

(2) - " for general type of reaction -as certain a.a. deaminases.

- Activity of all enzymes results directly from specific structure of enzyme protein component.

2/25/41)

Review :-

Bacterial enzymes:-

1. Constitutive
2. Adaptive

Bacterial mutation :-

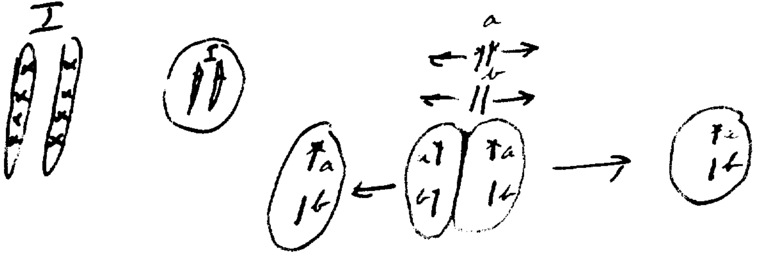
B. Coli :- fermentation of xylose (enzyme mutation form)

- mutation is relatively constant & is transferred thru several generations of B. Coli.

Chromosomes :- subdivided into genes.

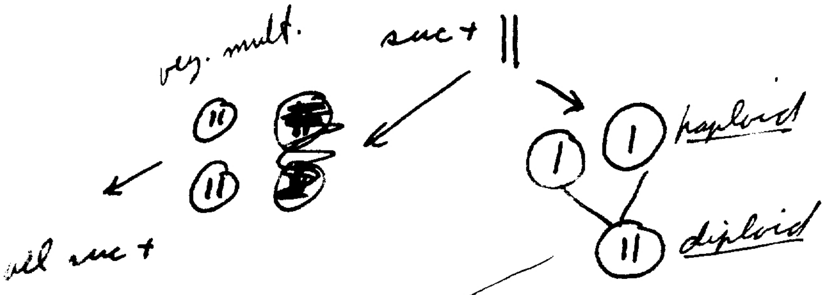
Each gene giving organism a specific characteristic

chromosomes occur in pairs in a cell :-



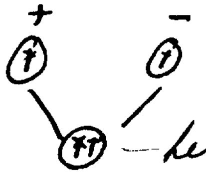
- cell which has 2 of ~~same~~ each chromosomes = diploid
" " " only one " = haploid

diploid



suc + = sucrose ferm. due to prod. of enzyme.

Some yeasts ferment sucrose
" " can't "



heterozygous - this heterozygous yeast cell will be able to prod enzyme which ferments sucrose

Homologous chromosomes

C ‡ ‡ homozygous (for suc.+)

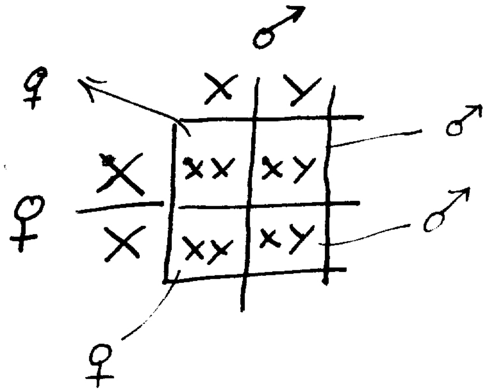
C † † " (for suc-

The prod of enzyme property is dominant in the heterozygous cell.

(41.

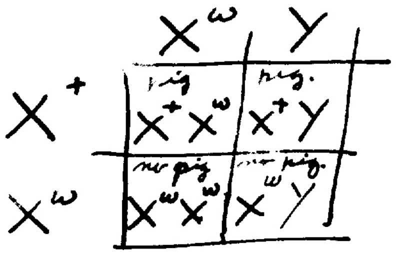
Haploid type

X · Y



	X	Y
X	XX	XY
X	XX	XY

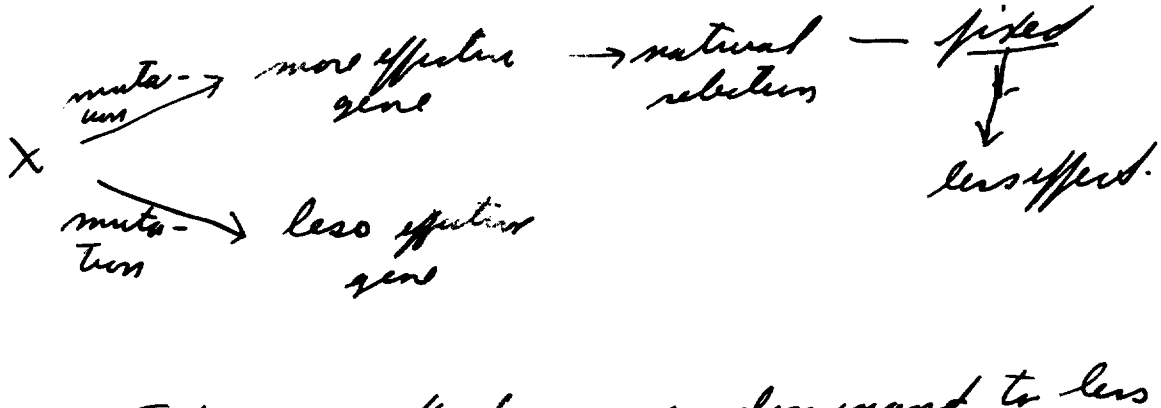
Y = male
X = female.



	X^w	Y
X^+	pig. X^+X^w	pig. X^+Y
X^w	no pig. X^wX^w	no pig. X^wY

+ = pigment eyes
w = white eyes
+ is predominant
w = recessive

Genes effecting metabolic processes :-



The more effect. gene is dominant to less effective gene.

In the case of carbhyd. metabolism in man:-

recessive   sugar (diabetes)

(– –) two genes forming a cell incapable of metab. sugar.

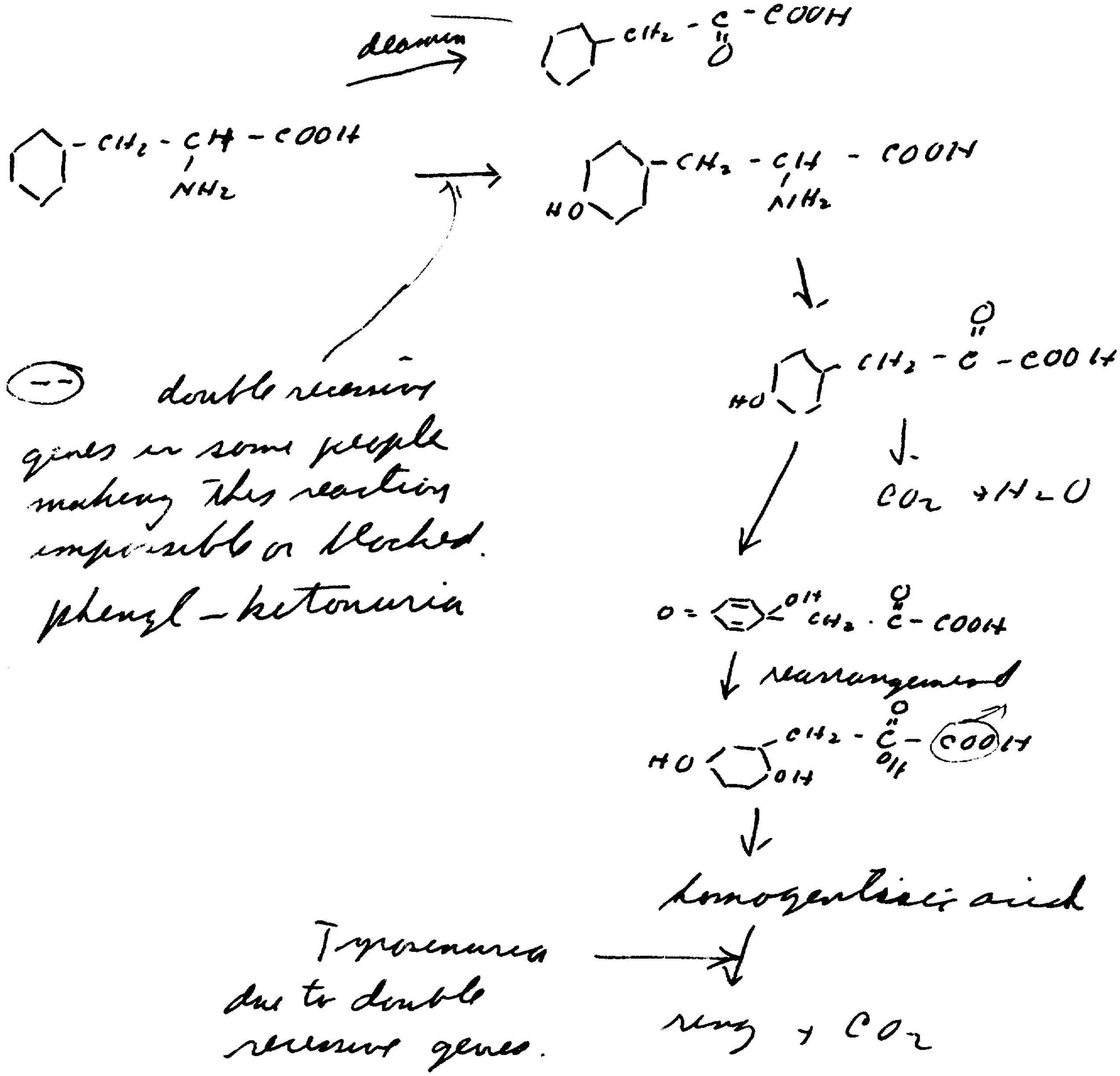
(OVER)

recessive

pentosuria

(- -) (double recessive genes)

y-ferm. sucrose

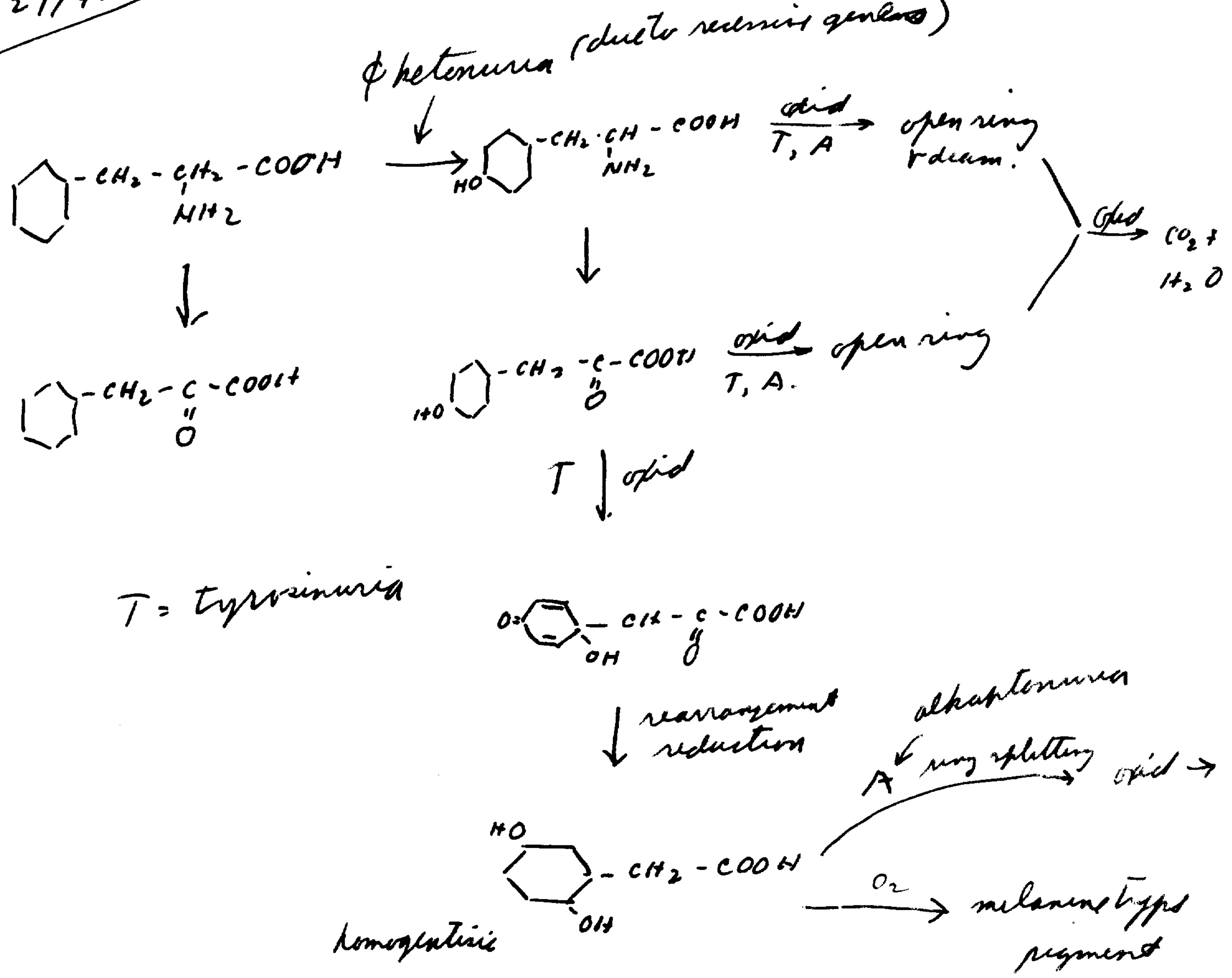


(- -) double recessive genes in some people making this reaction impossible or blocked.

phenyl-ketonuria

Tyrosinuria due to double recessive genes.

2/27/41



Above reactions may be blocked with incomplete metabolism due to presence of recessive genes.

Cystinurea :- involves metabolism of methionine

$$\begin{tikzcd}
\text{methionine} \arrow[r] & \text{cystine} \arrow[r] \arrow[d] & \text{cysteine} \arrow[dr, "\text{-OK}"] & \\
& \text{deposited in body} & \text{blocked by double recessive genes.} \arrow[ul, phantom, ""] &
\end{tikzcd}$$

Porphyrinuria :-

— o —

Uric acid is excreted by man, ape & Dalmatian dog.

- other mammals :-

uric a. $\xrightarrow[\text{uricase}]{O}$ allantoin

~~a = allantoinase~~

~~+~~

— o —

Hemophilia → less ability of blood to clot.

+ = normal
a = recessive

$\begin{pmatrix}+\\a\end{pmatrix}$ → normal clot

$\begin{pmatrix}a\\a\end{pmatrix}$ → double recessive
homozygous - neg blood clot.

(continued next page)

Clotting mech:-

$$\begin{tikzcd} K \arrow[r] & \text{prothrombin} \arrow[dr] & & \\ & & \text{fibrinogen} \arrow[r] & \text{fibrin} \end{tikzcd}$$

euglobulin fraction (++) → normal clot

- apparently euglobulin fraction synthesis is tied up with above normal clotting mechanism

— o —

There are 4 blood groups:-

agglutinogens

A A
A B → 1
O O → neither
B B = [illegible]
A O = Ⓐ

— o —

Chlorophyl syntheses:-

(aa) double recessive genes - block chlorophyll synth.

- there are any one of a number of genes which may be (aa) & block chlorophyll synthesis.

Storing of xanthophylls in rabbit fat cells

- destruction of xanthophylls in rabbit fat cells is dominant. Non destruction is recessive

— o —

Genetic characters of a certain corn; "nana"

nana - dwarf corn

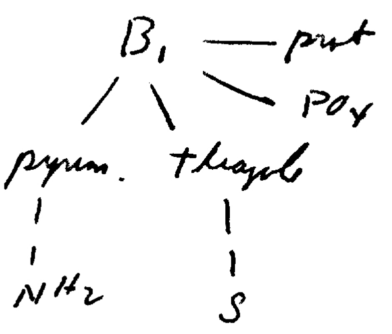
- morphological condition due to auxin (necessary for elongation of cells)
- auxin presence or absence depends upon genes

— o —

How genes effect or are involved in chem reactions

(a) - multiple genes effecting same reactions

such as in synthesis of B_1 ~~$B_1 \rightarrow$ prot~~ ~~$\rightarrow PO_4$~~

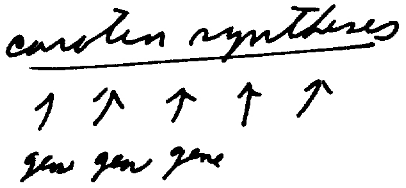


a chlorophyll synth

Chlorophyll
genes - ↑
genes - ↑
genes - ↑
genes - ↑

(b) - many genes having an accumulative effect (each gene responsible for prod of small am't of subst.)

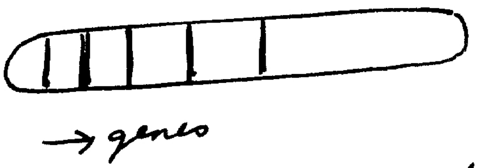
example :-



(c) - One gene responsible for complete series / reactions to specific product.

What do genes do?

- How do genes bring about certain types of reactions?
- Starting with gene itself :-
  - can be seen microscopically
  - chromosome



- much material of chromosome is nucleoprotein
  basic a.a.
  nucleic a.
- u. violet absorption is conc. in above bands (genes) of chromosome. Here is where nucleoproteins are concentrated

- Viruses, phage + genes are very similar in chemical composition :-
    - all high in basic a.a. content
    - max. inactivation of phage 2600 Å
        - same as max abs. of nucleoproteins & genes.
    - absorption of diff. viruses is about 2600 Å
    - similarity of size of virus :- 250 mμ
      phage :- "
      genes :- "
    - all can reproduce - existence depends upon living forms or organisms

3/4/41 Invertebrate pigments - Lederer
Biol. Rev. 15, 273 '40

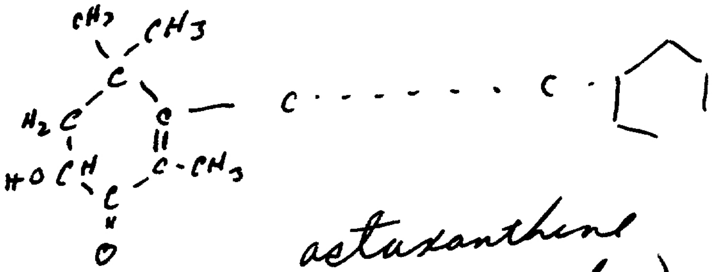
- have unknown attractive & repulsive powers or forces - formation of aggregates (virus)
    - attractive & repulsive forces of genes & chromosomes
- all can mutate (change specific structure)
    - mutation due to ionization effects
    - mutation due to irradiation x-ray etc - one mutation / quantum

- changes or mutations in genes
  - gene molecule altered (polymerization, isomerization etc.

— o —

<u>Starting with End Products</u> (<u>as means of characterizing viruses, phages & genes</u>).

Pigments found:-
- carotenoids (eggs & sex glands of invertebrates)



astaxanthine
(red-orange color)

↓ oxid

asticine

protein + ~~astaxanthine~~ asticine (greenish)

↓↑ 70° Δ

red.

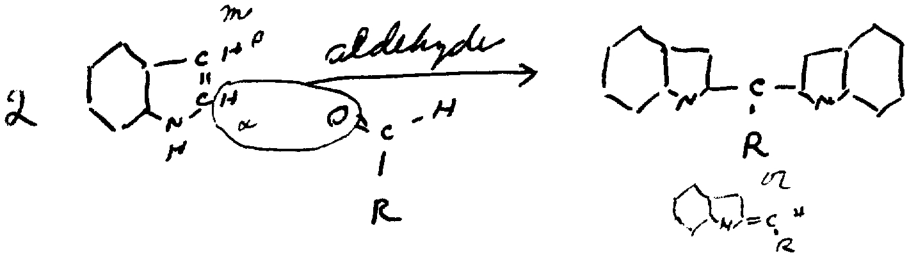
- pyrrole pigments (chlorophyll etc)
- pterines - 3 purine rings
- variety of miscellaneous pigments
  indigo etc.
- melanins (most important pigment

from a genetical pt. of view).

- are soluble in alkalies.

- More about melanins

(1) Chem. prep:-



if aromatic aldehyde used:-
prod. is stable + crystallizable
(such as p.di Me NH_2 Φ CHO)
pH indicator)

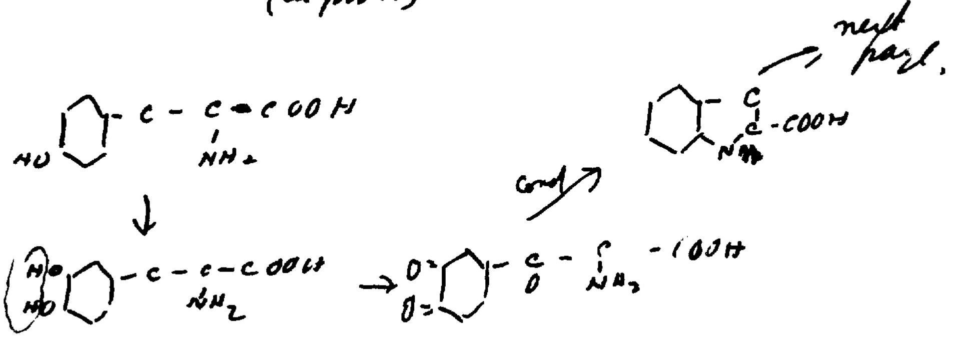
if aliphatic aldehyde used:-
prod. tends to polymerize

(2) - Photosynthetic melanins

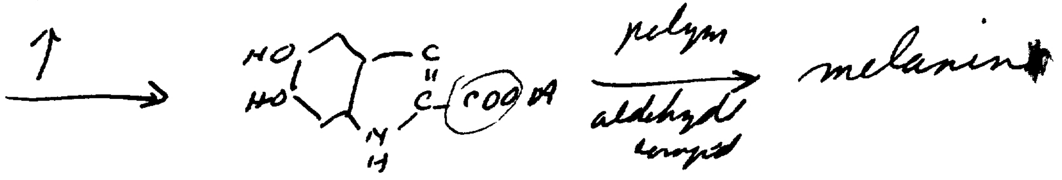
u.v on $\underbrace{\Phi alan}_{neutral} - \underbrace{Tyrosine \;\; - trypt.}_{alkaline\ soln.}$

(3) Biologically significant production:-

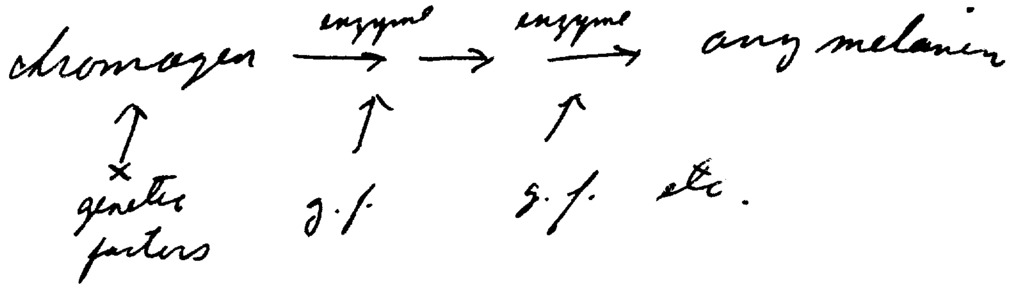
tyrosine $\xrightarrow[\text{(Cu prot.)}]{\text{tyrosinase}}$ melanin



hallachrome



Genetic control of these types of reactions forming melanins:-



X - dominant form - produce enzyme

o - recessive " - not " enzyme

Xo - heterozygous form . produces enzyme

Example of genetical analyses involving chemical reactions (melanin prod.)

Himalayan Rabbit :-

AA concerned with oxidizing enzyme production

aa no or very little " " "

Extract skin of animal

extract $\xrightarrow{\text{soln dopa}}$ AA > aa

(OVER)

case 1:-

pigments – only in tips of extremities (ears etc

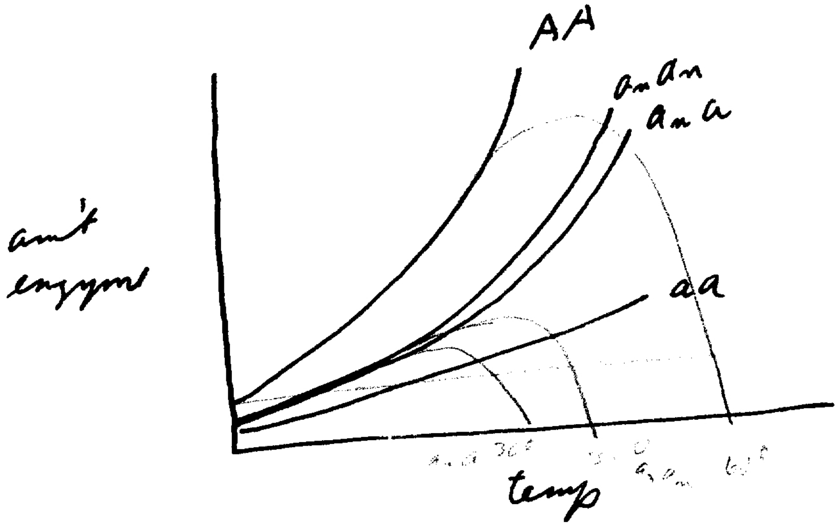
subject animal to temp > 35° no pigment

< 33° - pigment produced

Such animals have

$a_n a_n$ genes - intermediate am't of oxidizing enzyme prod.

$a_n a$ genes also



Increase temp & get increase rate of inactivation

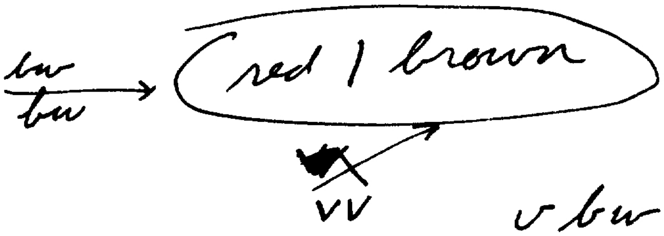
3/6/41

A = enzyme
a_n = less enzyme
a = no enzyme

$$AA > Aa > a_n a_n > a_n a > aa$$

Insect pigments

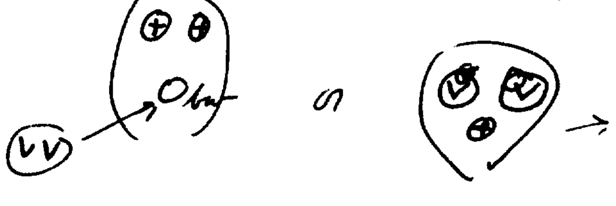
+ wild insect :- normal pigment is red + brown



v bw = white

v v = vermilion
bw = brown

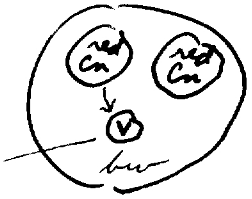
+ wild type eye (~~[illegible]~~ red brown)



→ host eyes become brown red upon injection of brown red eye.

non-autonomous

cn - $\frac{\text{cinnabar}}{\underline{\text{red}}}$



$$\begin{tikzcd} {} \arrow[r, "\underline{v^+}"] & v^+ \arrow[r, "\underline{cn^+}"] & cn^+ \arrow[r] & \text{bw pig.} \\ \underline{v} \arrow[u] & \underline{cn} \arrow[u] & & \end{tikzcd}$$

* * *

$v\ (-v^+)$

semi starvation → bw

→ resulted in production of v^+ hormone

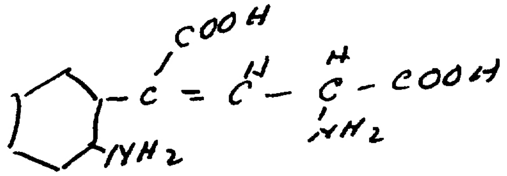
+ tryptophane to $\underline{v}$ → $\underline{\text{bw}}$

– but under aseptic conditions trypt effect was negative

(trypt + microorg) are necessary to replace v^+ hormone.

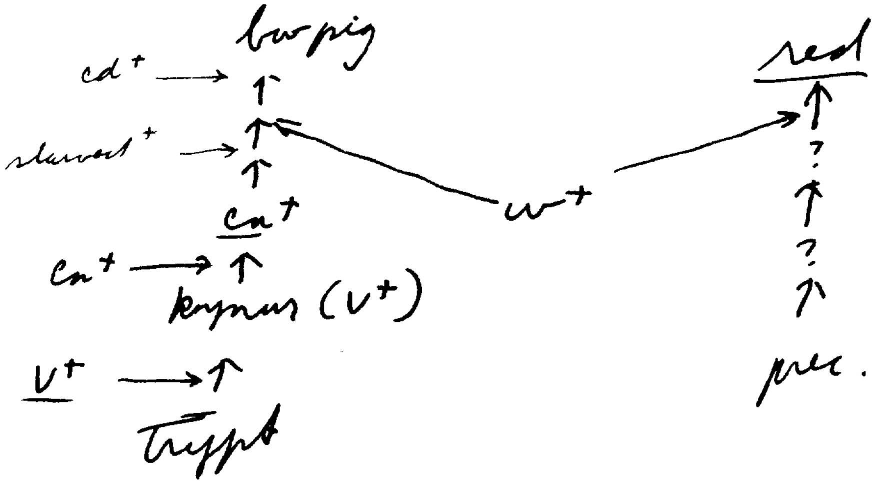
(trypt + microorg) → bw

V^+ substance is derived from tryptophane :-



kynurenine

— o —



w genes

w^+

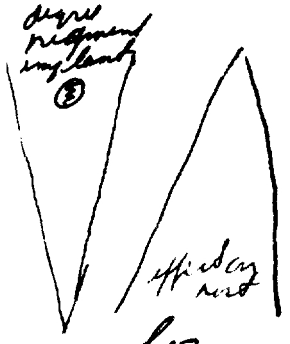
w^1

w^2

w^3

w^4

w — no color



Nature of the pigments:-

bw (brown) pig - insoluble (H_2O)
- oxidized - alk. soln.
- soluble acid alcohol.

$[O] \swarrow$ y-colorless

This pigment may be melanoid in character

---

red pig - completely H_2O soluble
- may be a pterine contain-ing 3 purines

$$\text{colorless} \xleftarrow{[H]} \text{red pig} \xrightarrow{[O]} \text{colored}$$

$$\text{yellow acid} \leftarrow \text{pH} \rightarrow \text{red alk}$$

3/11/41

Scott - Moncrieff - Jour. Genetics, 32, 117 (1936)

Control of Pigments in Insects - review

- controlled by genes
- can be modified by experimental condition
- chem changes as regards insect pigments are brought about genetically
- Reactions rather than precursors of pigments are controlled by genes (actually enzymes which catalyze reactions)

- Dominant gene has positive action (produces enzyme etc.
- Recessive gene has neg. action (no production of enzyme)

Example:-

V - no V^+ hormone (kynurenine)

$\underline{V^+}$ converted $cn^+ \rightarrow$ pig

cn - $\underline{V^+}$ no cn^+

w - $\underline{V^+}$ $\underline{cn^+}$ no pig

bw - no red has hormones

(OVER)

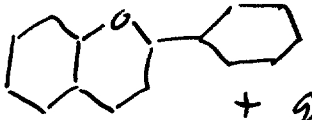
v bw = white

cn bw = white $\underline{v^{+}}$

v w — $v^{+} \rightarrow cn^{+}$

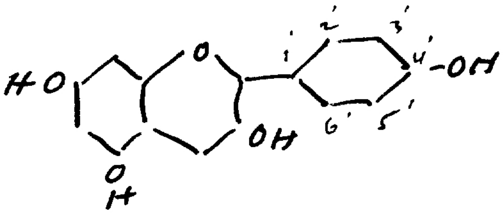
General nature of plant pigments, & the way they are controlled genetically.

Main type of pigment :- anthocyanins



+ glucose

- pelargonidin :-



anthocyanins usually occur in plants as glucosides

- colors of anthocyanin cover blue to red.

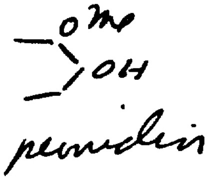
- anthocyanins $\xrightarrow{hyd}$ anthocyanidin + glucose or some other sugar.

Classification of plant pigments (anthocyanins), according to degree of blueness

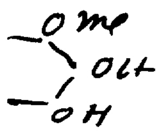
3', 4' di OH = cyanidin

3', 4', 5' tri OH = delphinidin

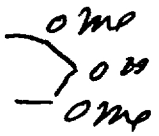
$\xrightarrow{ox}$ more blue



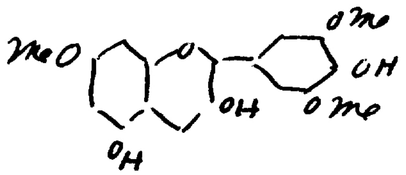
peonidin



petunidin



malvidin



hirsutidin

Increase methylation $\longrightarrow$ less blue

Factors controlling pigment

(1) - increased blueness by oxidation

(2) - decreased blueness with methylation

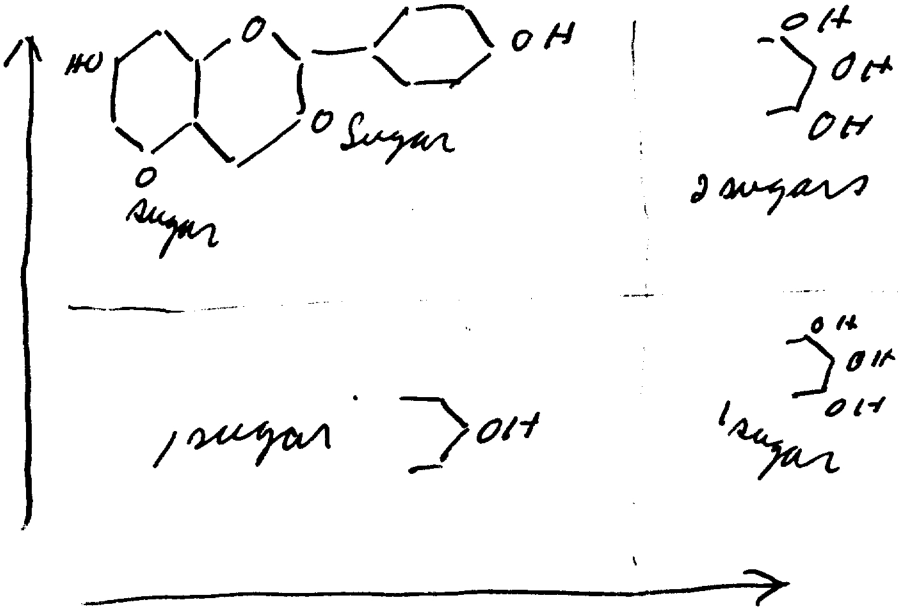
(3) - sugar residues increase blueness

(4) - pH effects color & intensity of color.

(5) - Co-pigmentation intensifies blue (anthoxanthins)

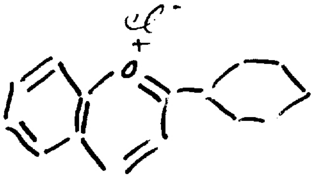
(6) - Colloidal state

- verbenas



- blueness increases in direction of arrows.

Effect of pH on color :-



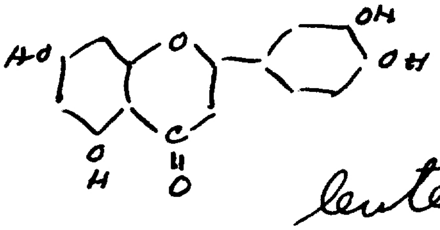
acid -	neutral	Na OH
oxonium salt red	inter-mediate	blue

Other types of pigments in plants

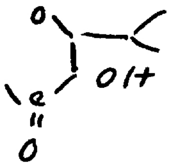
carotenoids

xanthophylls

anthoxanthins: —



luteolin



quercitin

- very much less intense in color
    - ivory to yellow
- the ivory varieties have marked effect on anthocyanins. This is called co-pigmentation (intensifies blueness of an anthocyanin).

Correlation of pigment controlling factors + genetic control :-

~~dominant~~
~~control general production production~~
~~(1) incr Bl by oxid~~
~~(2)~~
~~(3)~~
~~(4)~~

dominant

- control general production – productions
- specific prod. — production

(1) incr Bl. by oxid — oxid
(2) decr Bl. with Me — methylation
(3) sugar res. - intens — complexity & no sugar residues
(4) pH change — lower pH production (incr. organic acids)
(5) Co-pigmentation — prod anthoxanthins

There is a competition effect between anthocyanins & anthoxanthins which is controlled genetically

3/13/41

General Review

- Methods of obtaining energy: -
  - evolutionary development
  - mechanisms + reactions involved
    - respiratory mediators
      - similarity of mechanisms in various scales of life.
      - nucleotides, coenzymes, enzymes, C_4 dibasic acids etc.
  - analogies of amino acid + carbohydrate catabolism
  - energy obtained + its utilization for life
    - growth
    - restorations of cell material, hence syntheses
- Syntheses by cell
  - cbhyds, a.a., proteins etc
  - however, some things can't be synthesized by organisms (vitamins etc.). This represents a progressive loss of power to synthesize by the organism

– Vitamins probably function as catalysts

– ability to synthesize a.a.
  – nature of essential a.a.
  – " " none " a.a.

Validity of assumption that all metabolic processes are essentially the same or related

Structural characteristics of organisms
Reactions " " "

– Both are controlled by specific enzymes

Enzymes:– adaptive
specific

– controlled genetically

Some aspects of gene action
– similarity to viruses & phage
– size, composition of protein and of viruses, phage & genes.

– cause of mutations

Types of reactions which can be modified or controlled genetically.

- vital modifications of metabolism
- non-vital " of, say, pigments

Genes have a positive effect

- dominant gene
  - prod. of enzyme etc.

- mutation of genes involves loss of a function - recessive genes

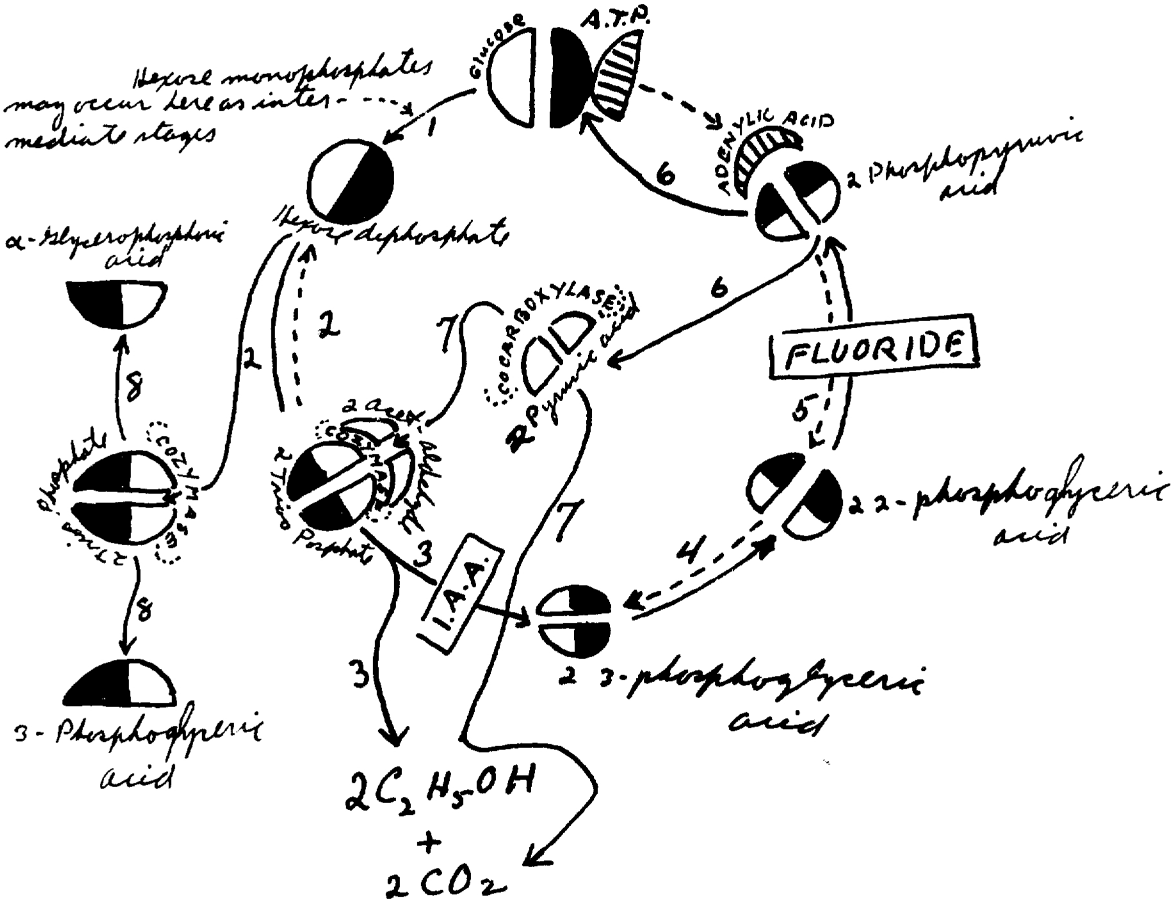
General nature of action of genes.

- blocking of reactions
- a way of arriving at actual no. of chemical steps in a synthetic process.

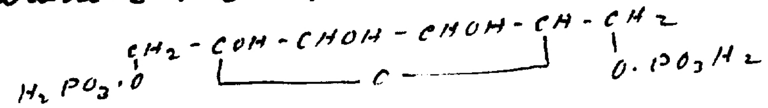
Assume that all processes in cells are genetically controlled.

Current Theory of Fermentation of Hexoses
- Embden, Meyerhof & Parnas

Glucose



Glucose
Fructo-furanose 1:6-diphosphate :-



$$\underset{H_2PO_3\cdot O}{CH_2}-\underbrace{COH-CHOH-CHOH-CH}_{O}-\underset{O\cdot PO_3H_2}{CH_2}$$

Dihydroxy acetone phosphate :-

$$\begin{array}{l} CH_2OH \\ | \\ CO \\ | \\ CH_2O(PO_3H_2) \end{array}$$

3-Phosphoglyceric acid :- $CH_2O(PO_3H_2)CHOH\cdot COOH$

2- " " :- $CH_2OH\cdot CHO(PO_3H_2)\cdot COOH$

Phosphopyruvic acid :- $CH_2=CO(PO_3H_2)-COOH$

Pyruvic acid :- $CH_3\cdot CO\cdot COOH \rightleftharpoons CH_2=COH-COOH$

Acetaldehyde :- CH_3CHO

Alcohol :. C_2H_5-OH

Carbon Dioxide :- CO_2

Starting at the point where glucose enters the machine the first action is that of phosphorylation :-

1. Glucose + ATP = fructose diphosphate + adenylic acid

The hexose molecule now remains phosphorylated till the final stage of degradation is reached and the intramolecular changes and oxido-reductions all occur in phosphorylated molecules.

2. Fructose diphosphate → 2 triosphosphate
(2 dihydroxyacetone phosphate)

3. Dihydroxyacetonphosphate + acetaldehyde →
ALCOHOL + 3-phosphoglyceric acid
(the coenzyme I reaction)

4. 3-Phosphoglyceric acid → 2 phosphoglyceric acid

5. 2-Phosphoglyceric acid → phosphopyruvic acid.

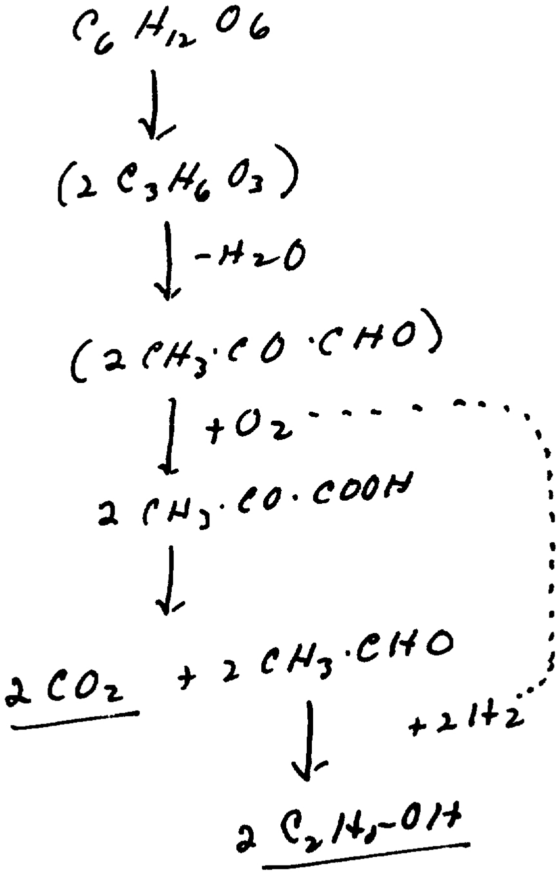
6. 2-Phosphopyruvic acid + adenylic acid → A.T.P.
+ 2 pyruvic acid

7. Pyruvic acid → CARBON DIOXIDE +
+ acetaldehyde (co-carboxylase reaction)
↓
To equation (3)

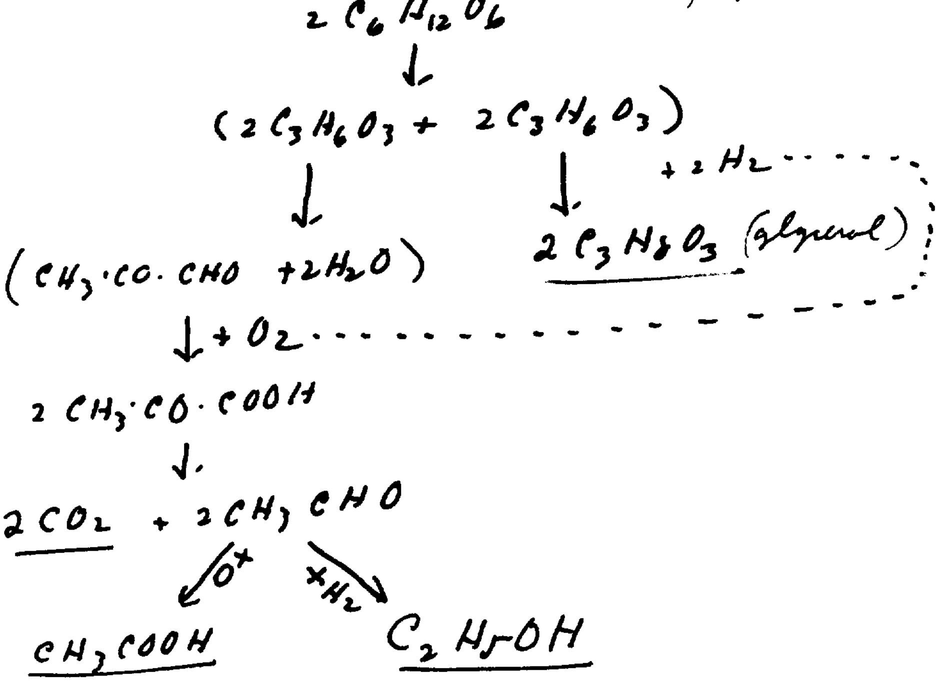
8. 2 Triose phosphate → glycerol phosphate
+ phosphoglyceric acid

Earlier work by Neuberg: -

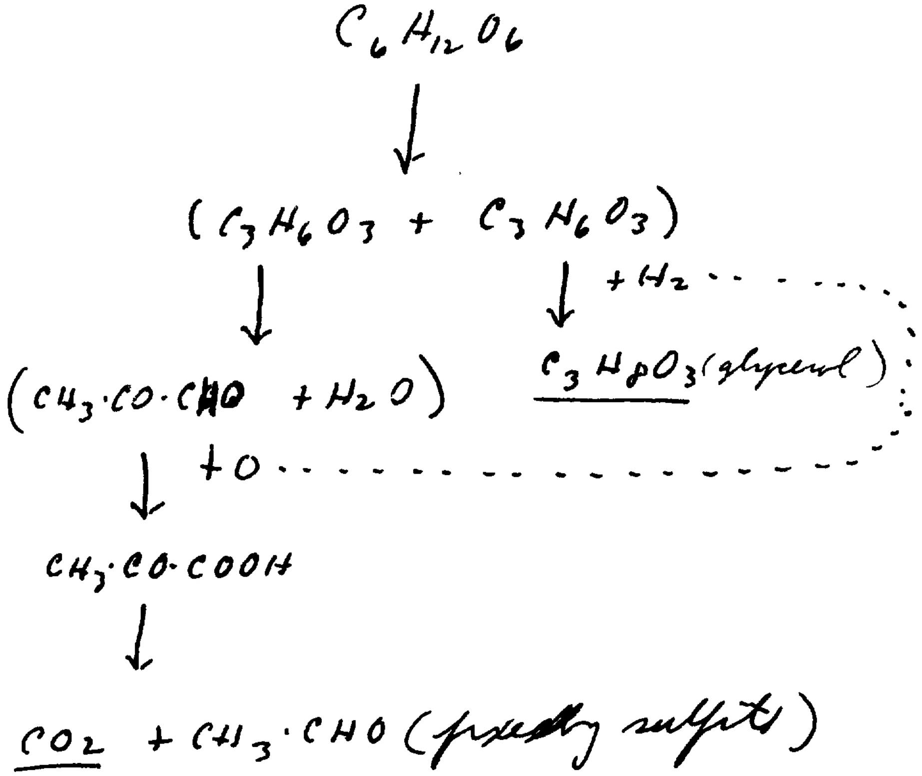
Scheme I (Neubergs "first form" of fermentation)



Scheme II - If reaction of medium is alkaline (Neubergs "third form" of fermentation)

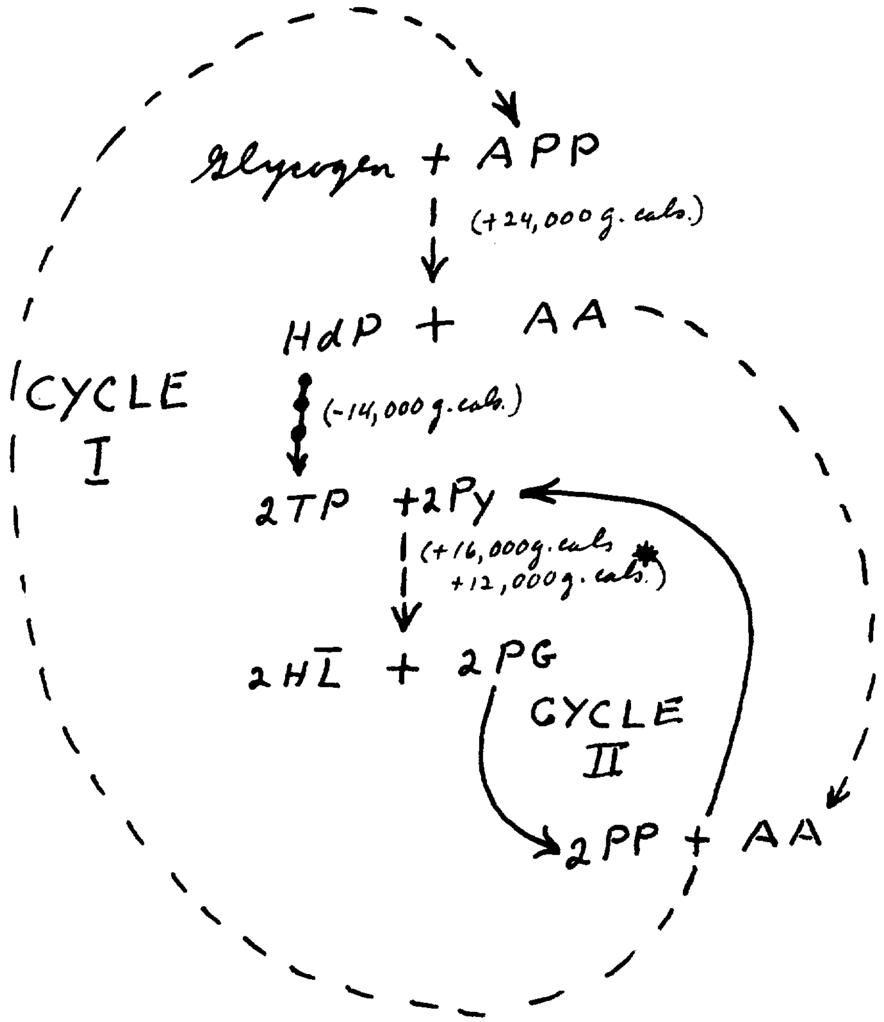


Scheme III (Neubergs "second or fixation form" of fermentation)



Chemical Cycles in Muscle Contraction

Scheme I



APP = adenylpyrophosphate
HdP = hexosediphosphate
AA = adenylic acid
TP = triosephosphate

Py = pyruvic acid
HL = lactic acid
PG = phosphoglyceric acid
PP = phosphopyruvic acid

* This 12,000 g. cals. is due to neutralization by protein of the acid formed.

Adenylpyrophosphate + 2 creatine ⇄ adenylic acid
+ 2 creatinephosphate

Here the heat provided by hydrolysis of one molecule of adenylpyrophosphate is just about equivalent to that needed for synthesis of two molecules of creatinephosphate - the reaction is thermally neutral.

$$\text{Adenylpyrophosphate} \xrightarrow[\text{phosphatase}]{\text{adenylpyro}} \text{AA} + \text{orthophosphate}$$

—— o ——

In presence of fluoride:

$$\text{Glycogen} + 2\,PP + 2H_3PO_4 \rightarrow 1\,HdP + 2\,PG + 2\,HL$$

In presence of iodoacetate:

$$\text{Glycogen} + 2\,PP \rightarrow HdP + 2\,Py$$

Scheme II - (for happenings during anaerobic recovery).

I. Glycogen + 2 H_3PO_4 → 2 hexosemonophosphate

II 2 hexosemonophosphate + 2 adenylpyrophosphate →
2 hexosediphosphate + adenylic acid (+ 24,000 g. cals.)

III 2 hexosediphosphate $\rightleftharpoons$ 4 triosephosphate
(-28,000 g. cals.)

IV 4 triosephosphate + 4 pyruvate → 4 phosphoglycerate
+ 4 lactate (+ 72,000 gr. cal + c. 24,000 g. cals.)

V 4 phosphoglycerate → 4 phosphopyruvate (± 0 g. cals.)

VI 2 phosphopyruvate + adenylic acid → 2 pyruvate
+ adenylpyrophosphate (-7400 g. cals.)

VII Adenylpyrophosphate + 2 creatine → 2 creatinephosphate
+ adenylic acid (+1000 gm. cals.)

VIII 2 phosphopyruvate + adenylic acid → 2 pyruvate
+ adenylpyrophosphate (-7400 g. cals)