ALL GLORIES COME FROM DARING TO BEGIN ……

READY REFERENCE PATTERN

(SCRIPTS AND TRUMP CARDS)

An Innovative Method Designed, Blended and Executed By

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…..WHEN THE GOING GETS TOUGH, THE TOUGH GETS GOING

HIGHLIGHTS

- More organized, well planned research way of studying.
- Collection of perfect, precise, point-wise and paragraphic information from various text books, research journals, reference books, encyclopedia, websites etc.
- Screening of target information with maximum illustrations.

BENEFITS

- Develops curiosity of learning.
- Enables the students to believe and remember principles, scientific laws, facts etc. established by others.
- Inculcate values of learning process.
- Builds up proper base for professional & higher educational courses.
- Allows students to interact with others during learning process.
- Increases confidence required to face the academic challenges.
- Facilitates ones understanding process and makes it easier for learners to remember.
- Encourages learners to quote references systematically inculcating values of acknowledging the work of others.

…ALL'S WELL THAT ENDS WELL

GOVT. COLLEGE OF ARTS AND SCIENCE
AURANGABAD.

READY REFERENCE PATTERN

• SCRIPTS •

• MICROBIOLOGY •

NAME : Ms Hoimee Himadri Dey.

CLASS : B. Sc. IInd yr. R. NO. 69

SCRIPTS ENCLOSED :

- Fermentors & Fermentation media.
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2003-2004

Script no-1

Fermenters & Fermentation media.

Hoimee Dey.
BSc IInd yr.

• 2003-2004 •

Date :

Index :-

Date : 18th Nov '03

1.1 Introduction :-

- Fermentation technology is a widely applied stream of biology. It shares the same pedestral with other upcomming streams.
- The technology finds its relevance in all types of bio-industries such as beverage industries, refineries etc.
- Proper working of these, hence, depends on a planned and scheduled work layout.
- Fermentors, being an important part of the system, is designed according to specific requirments of every reaction. The considered issue ranges from economic condition, environmental condition to the many facets of biosynthesis.
- Even the nutrient media is created inorder to suffice all the required criterion for maximum and healthy yield and minimal cost rates.
- The following chapter will enable us to commemorate every single detail regarding

"Fermenters and Fermentation media."

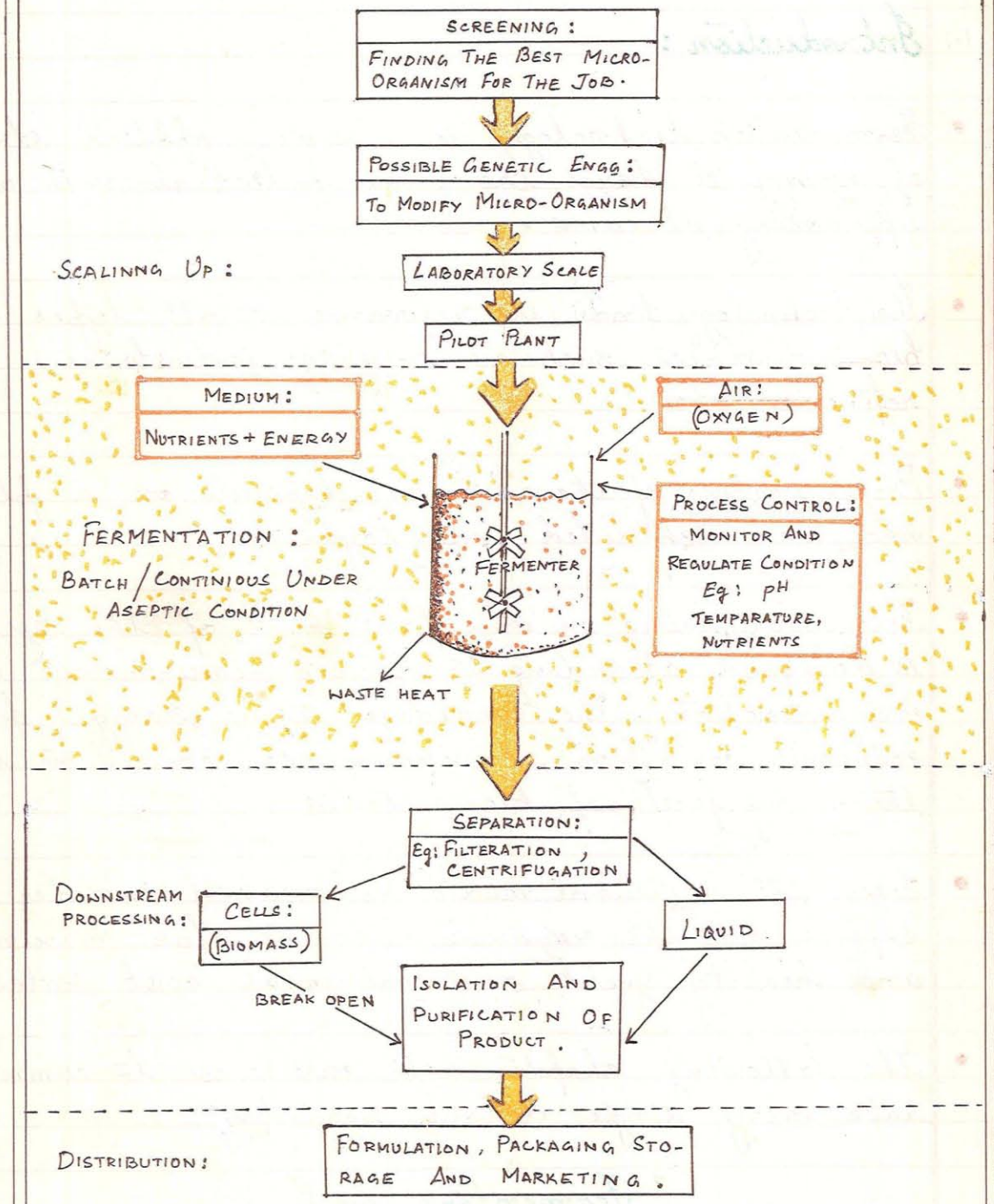


Fig 1.1 Overview of a biotechnological process.

Taylor (3Ed); 393.

SELECTION OF APPROPRIATE STRAIN OF A PARTICULAR SPECIE OF MICRO-ORGANISM: Determines the product yielding phase of growth; temparature; pH range; degree of required aerobiocity and the effect of contdmination.

↓

SELECTION OF APPROPRIATE FERMENTOR-CONFIGURATION

↓

DETERMINATION OF FERMENTOR DIMENTION: Volume and Diameter; Operating variables; Concentration; Temparature and pH; Process line for batch fermentation & Flow rate with continious fermentation

↓

EXTENT OF HEAT TRANSPER SURPACE AND MIXING DEVICES

↓

POWER AND AERATION REQUIRED

↓

MECHANICAL DESIGN: selection of constructing media and maintenance of aseptic condition.

↓

MONITORING AND CONTROL

↓

SAFETY FACTORS

Fig 1.2 Flow chart representing "Fermentor-designing".

Date :

1.2 Fermenters :-

Fermenters are specially designed vessels loaded with perticular type of nutritive media used for growing microbes in fermentation industries.

They are complicated in design, since they must provide for the control and observation observation of many facets of microbial growth and biosynthesis. The design of fermenters depends on the purpose for which it is utilized. Some specifically designed fermenter are the submerged used in laboratory, semi pilot plant & pilot plant scale.

1.2.1 Fermenter designing :-

The fermenters must be properly and specifically designed for each purpose. Fig 1.2.

1.2.1.1 Factors influencing fermenter design :-

According to Levenspiel (1971), the two basics of fermenter designing are :-

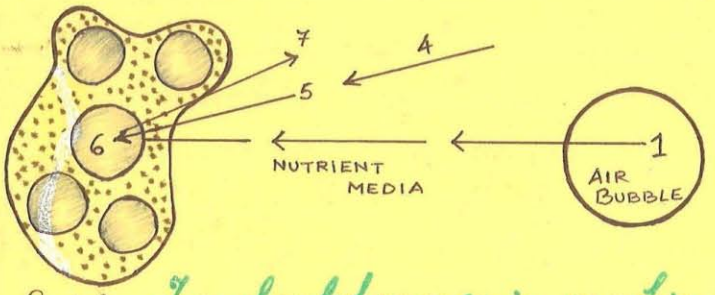
- Selection of best reactor for a perticular type of reaction.
- determination of best operating condition.

1.2.1.2 Objectives of design :-

- describe the effect of operating condition on performance a bioreactor.
- comparison of alternative design with economic criteria.

Sl No.	Character	Biochemical Conversn	Chemical Conversn
•	Reactant Mixture	Complex	Relatively simple
•	Microbial Mass	Increases with the accomplishment of reaction	No such phenomenon observed.
•	Catalyst (Enzymes)	Autosynthesised	External chemical catalyst required
•	Temparature & pH	Relatively mild	Variable
•	Stability	Difficult in maintaining	Varies with the nature of reaction
•	Phase	Restricted to aqueous phase	No such restrictions
•	Substrate/ Product Concentran	Relatively low	High.

1.2.1.3 Difference between biochemical & chemical process



1. Oxygen absorbed by aqueous phase
2. O_2 transfered through aq.. phase
3. O_2 absorbed by & transfered through intercellular gel to rxn zone
4. Org.. substrate transfered through aqueous phase.
5. Org.. substance absorbed & transfered though intercellular gel to reaction zone.
6. Microbial reaction zone.
7. Product transfered to aq phase

Fig 1.3. Transport process in aerobic fermentation

Date :

1.2.1.4 **Rate processes :-**

The overall rates are influenced both by the reactants & the products (autocatalysis). This leads to rather unusual optimisation problems.

- Essentially all configurations of microbiological reactors consist of microbes dispersed in aqueous nutrient media.
- In aerobic reactions an additional dispersed phase consisting of air bubbles (Fig:) is present. The overall rate of reaction depends upon the absorption of oxygen.
- Secondary metabolites include carbondioxid & other products.

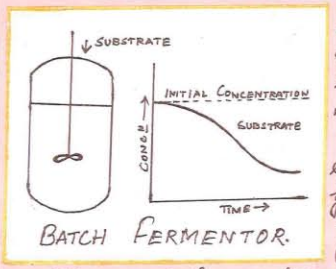
1.2.1.5 **Operational considerations :-**

- In a particular system all the flowing molecules neither have same residence time, nor the same history of temparature & concentration.
- Even in case of time invariant state of system, transients are equally important.
  - calculation & minimisation of time required for start-up procedure.
  - investigation of product time at the approach of start up
  - calculation of fluctuation speed at the inlet, outlet or any intermediate point.

1.2.1.6 **Local conditions :-**

For any reaction the conversion depends on the

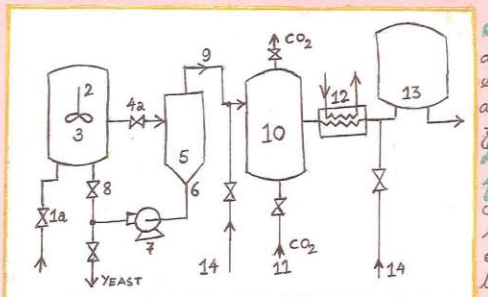
The Batch Fermentor.



BATCH FERMENTOR.

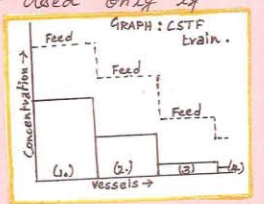
Size :- Varying range. Lab scale 1-2 lt max. 15 lt Pilot plant 25-100 gallons max: 2000 gallons Largest 1,00,000 gallons. Horton sphere :- Spherical batch fermentor 2,50,000 - 5,00,000 gallons. (1/5-1/4 of the fermentor is left vacant for aeration etc - Head space).
pH control :- acid-alkali addn ; autolitration titrator connected to pH probe. used only if reqd.
Temperature control :- Used only if required; is achieved by water jacket / supplementedly by coils.
Agitation :- It consist of a shaft to which impellers that are inturn mounted with blades are fitted. In some blades of diff function are used.
Aeration :- Sparger fitted with 1/64 - 1/32" holes the air passed in is sterile & hence costly.
Time dependence :- Substrate, microbial mass depends on microbial mass and biochemical product concentration
Positional variation :- Substrate & product is completely mixed (ideally). Even microbial-mass concentration is completely mixed.
Environmental history :- Varies over course of fermentation process.
Features of industrial importance :- labour-intensive.
Chief industrial application :- Most commercial application.

The Continious Stirred tank Fr.



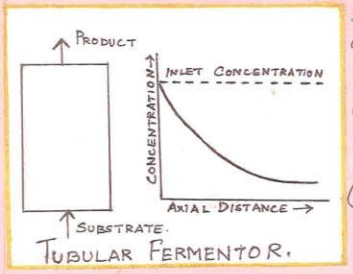
1. WORT INFLOW 1a. CONTROL VALVE 2. FERMENTOR 3. IMPELLER 4. BEER OUTFLOW 5. SEDIMENTATION VESSEL / CENTRIFUGE 6. YEAST RECIRCULATION 7. PUMP 8. RECIRCULATION CONTROL VALVE 9. CLEAR-BEER OUTFLOW 10. WASHING VESSEL 11. CO_2 12. HEAT EXCHANGER 13. RESERVOIR 14. FINING INLETS
FLOWCHART OF C.S.T.F.

Size :- Relatively smaller than batch fermentor
Distinctive feature :- In C.S.T.F, the contents of the vessels are at the steady st.. by using the chemostatic or turbostatic principles.
pH control :- Used only when required
Temperature control :- Used only if required.



Time dependence :- No.
Positional variation :- Substrate & product and even microbial mass concentration is completely mixed.
Environmental history :- Constant (all flows exposed to same environment)
Industrial importance :- Flow rate limited by wash out
Industrial application :- Waste water treatment, microbial protein production, alcohol production (beer), prodn of baker's yeast.

The Tubular Fermentor.



TUBULAR FERMENTOR.

State :- The microbial mass in a fermentor exist in two geometric state — i) freely suspended ii) surface adhesion

pH control :- Difficult (only if required).

temparature control :- Used if only required.

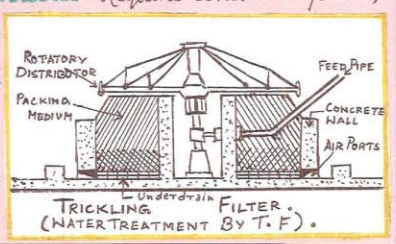
Time dependence :- In case of flocs it is time independent but in case of accumulation of microbial film slight change may occur. This will also effect substrate-product concentration

Positional variation :- Substrate & product and even microbial mass vary from inlet to outlet. In case of microbial film, mass is much independent of position

Environmental history :- (i) **floc:** varies as they travel through the fermentor (ii) **film:** constant film is exposed to different environment in different parts.

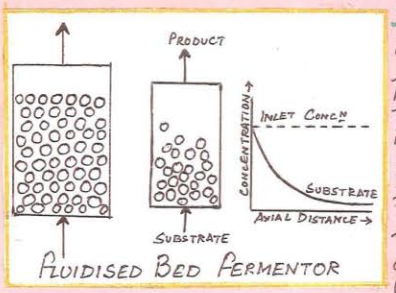
Industrial importance :- Requires constant feed, difficult hold up.

Industrial application :- Waste water treatment, vinegar production.



TRICKLING FILTER. (WATER TREATMENT BY T. F).

The Fluidised Bed Fermentor.



FLUIDISED BED FERMENTOR

Bed features :- i) Increase in porosity from bottom to top. ii) Decreased particle movement compared to beds of constant size particles (In case of the presence of gas phase particle distribution is uneven

pH control :- Difficult to control

temparature control :- Used only if and when required

Time dependence :- Independent

Positional variation :- Substrate & product and even microbial mass concentration varies from inlet to outlet.

Environmental history :- Largely constant, but some movement of flocs does take place as different parts are exposed to different milieu.

Industrial importance :- Flowrate limited by wash-out

Chief industrial application :- Production of alcohols such as beer and cider (sugar fermentation. Tower fermentor used for continious production of beed is modified FBF.

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following factors :-

- Residence time distribution.
- Concentration distribution.
- Temparature distribution.

1.2.2 **Classification of Fermentation processes :-**

Fermentors & there by fermentation processes are classified into four types :

- Batch fermentor
- Continious stirred tank fermentor
- Tubular fermentor
- Fluidised bed fermentor

1.2.2.1 **Other parts of fermentor system :-**

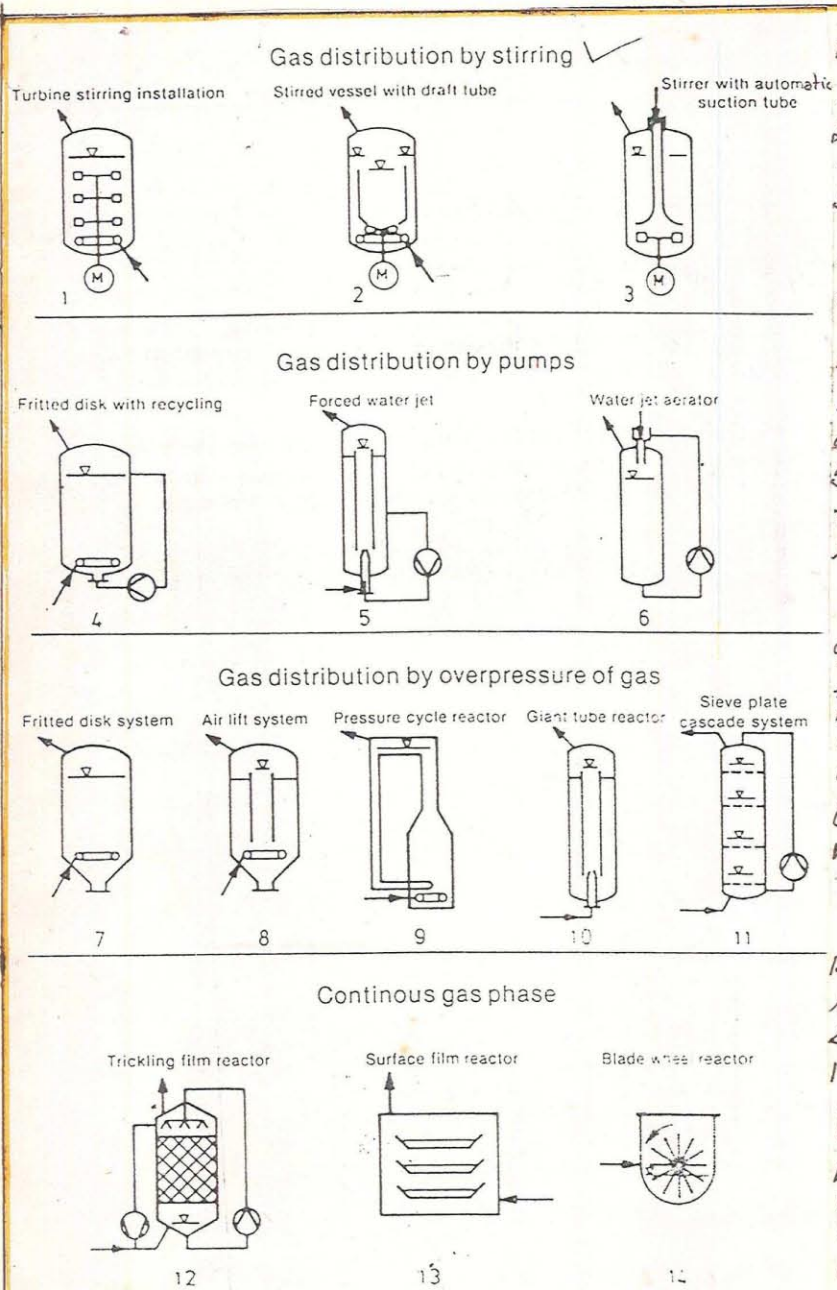
In a bioreactor or fermentor, production of metabolite must be accomplished with maximum emphasis on reliability for the process and minimum capital investment.

The reactors are hence designed specifically for special processes. Eg : **"Gas distribution"**.

In case of aerobic process gas distribution is of four types designed as per requirment

Gas distribution by stirring
Gas distribution through pumps
Gas distribution by means of pressurized air
Continious gas phase.

The distributory phase is illustrated & explained in



1. Stirred vessels are flexible & widely used
2. Loop reactors are mass productive
3. Automatic suction tube stirrer — vinegar production & water treatment

4. Modified air lift system — independent distribution.
5, 6. Direct mixing by water jet pump is most efficient.

9. Pressure cycle fermenter — first model of this kind — used for single cell protein prod^{n} Others mostly have no movable part.

12. Trickle-film reactor — org(s) grow on solid inert carrier.
13. Surface reactor — grow on semi-solid nutrient medium.
14. Blade wheel reactor — growth on blades
Waste water treatment Leaching ores, vinegar citric acid prod^{n} etc.

Fig 1.4 Types of bioreactors on the basis of gas distribution

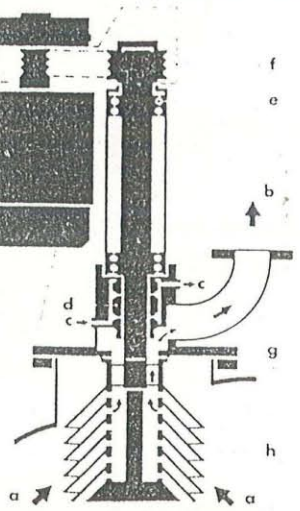


Fig 1·5 Mechanical foam separator (Fundafom, Chemap). a) Foam entrance, b) Gas exit, c) Lubrication, d) Double seal, e) Packing, f) Drive, g) Intermediate flange, h) Rotating plate

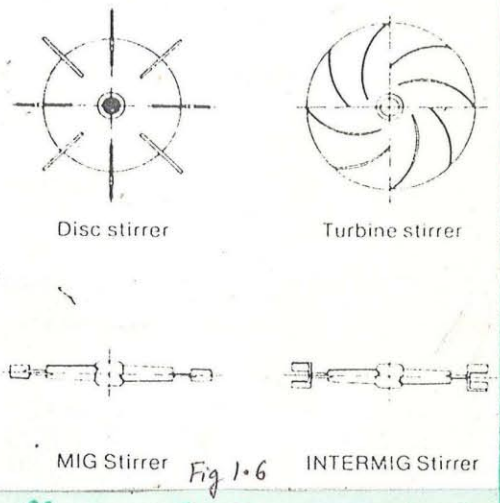


Fig 1·6

Types of Stirrer (4)

4

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- For industrial use in pharmaceuticals, most versatile bioreactors are the **"Simple Stirred, Aerated Fermentor"**. However no single single which adequately meets the needs of all biological system can be constructed. Laboratory fermenters are made of glass and are of 20 l volume. For larger ones the volume extends to 3000 l and are made of stainless steel. The height-width scale varies between 2:1 to 6:1 & stirrer may be top or bottom driven.
- In order to bring about turbulence to the fermentor wall **"baffles"** are used. Four baffles are commonly used with a width of 1:10 or 1:12 of the fermenter diameter. In large fermenters ($>100 m^3$) where heat dissipation is a problem, even as much as 12 baffles may be installed. They also help in reducing vortex.

Foaming is a typical problem in large scale aerated system. Simplest is the one with rakes mounted on stirrer. In Fring's system and Fundafom's system **"foam separation"** is done by centrifugal force.

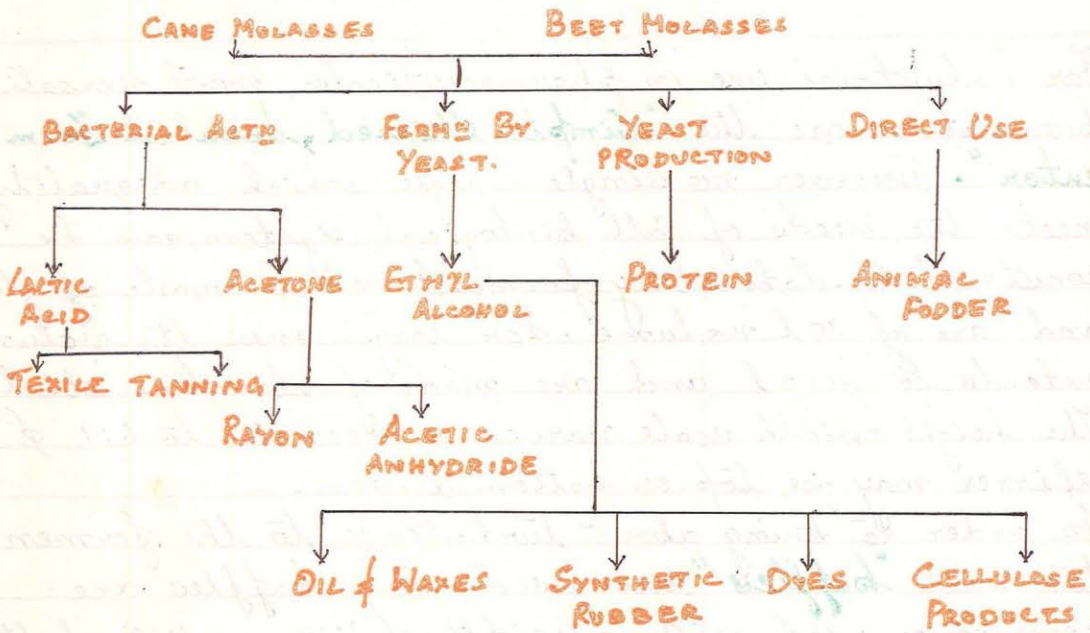
The **types of stirrers** used in microbial reactors are as below :-

Disc stirrer : 4-8 blades project out beyond the disc edge

Turbine stirrer : blades are curved; requires 50% less air for same yield & energy consumption

MIG Stirrer : 25% less energy consumed

INTERMIG : 40% less energy consumed.



Uses of Cane and Beet molasses.

Average composition of black strap molasses

Component	Percentage.
Water	20
Ash	8·0
Total sugar	40-60
Total nitrogen bodies	3·0
Total nitrogen	0·5
Gums	2·0
Free acid.	2·0
Combined acids.	3·0

Patel (1985) 45,47

Some fermented food products :

STARTING MATERIAL	PREDOMINANT ORGANISM	PRODUCT
• Cabbage	Leuconostoc mesenteroides Lactobacillus plantarum.	Sauerkraut
• Cucumber, tomatoes, lemon, cauliflower etc	Le. mesenteroides, La. plantarum, Le. brevis, Streptococcus faecalis, Pediococcus cerevisiae	Pickle
• Rice & black grams	Le. mesenteroides, Streptococcus faecalis P. cerevisiae.	
• Soybean, wheat and rice	Aspergillus oryzae, Hanensula and Saccharomyces	Soy sauce
• Rice	Mucor, Rhizopus and Yeast	Ragi
• Rice	Monascus purpureus	Ang-kak
• Soybean	Rhizopus oligosporus.	Tempeh
• Soybean	Bacillus subtilis	Natto
• Soybean, rice cereals etc	Aspergillus orizae Saccharomyces rouxii	Miso
• Beaf/pork	P. cerevisiae, P. acidilactici	Sausages

Date :

1.3 Fermentation media :-

1.3.1 Classification of Raw materials :-

Many different raw materials are used in fermentation industries. Mostly industrial products are used as raw materials but industrial wastes are of more biological importance as :-

- Produced in huge quantity.
- Have high B.O.D and hence are hazardous polluting agents
- Store high amount of usable nutrients. Also meet the increasing protein demand.
- Calorific value can be regained as biogas and ethanol.
- Renewable, thereby causing no substrate shortage
- Less expensive recovery.
- Not a part of human food chain.

1.3.1.1 Saccharine Material :-

Molasses : BY PRODUCT OF – Sugar cane and beet sugar.

NAME – Cane – blackstrap molasses.
Beet – beet molasses

FERMENTABLE % AGE – 95%

VITAMINS PRESENT – Biotin, pyridoxine, thiamine, pantothenic acid and inositol

FERMENTED PRODUCT – Spirit (ethyl alcohol), country liquors, rum, brandy, gin & whisky

Chemical composition of beet molasses (dry wt):-

Components :-	percentage (%)
Sucrose	48-50
Raffinose	1
Glucose + Fructose	1
Nitrogen compounds.	12-13
Glutamic acid	3.5
Other amino acids. (asparagine, aspartic acid, glycine, alanine)	5.5
Betaine.	3-4.25
Ash	11-12

Approximate chemical composition of beet molasses ash:-

Components :-	percentage (%)
K_2O	45
Na_2O	15
P_2O_5	0.7
CaO	3
MgO	1.8
SO_4^{2-}	6.3
Cl^-	18
Fe_2O_3	0.2
Al_2O_3	0.8
SiO_2	1.1

Fruit juices:-

Components :-	percentage.
N/R sugar	17%
Acid (D-tartaric with malic acid)	1%
Ash (mainly P_2 and K_2	0.3%

Date :

Fruit juices : SOURCE – Fruits (grapes, apples etc..)
PROVIDE – Carbon source to fermentation industries
FERMENTED PRODUCT – Grape wine, Apple juice etc.

Cheese whey : BY PRODUCT OF – Cheese
FERMENTED PRODUCT – Lactic acid, SCP, lactose vitamins (riboflavin)

Chemical composition of cheddar whey (Berry) :–

Components :–	Percentage (%)
Total solids	6·6 – 7·1
Proteins	0·82 – 0·95
Fat	0·12 – 0·36
Lactose	4·62 – 5·01
Ash	0·366 – 0·649
K	0·135
Ca	0·047
Mg	0·010
P	0·160

1·3·1·2 **Starchy Material :–**

SOURCE – Cereals (eg: wheat, rice, maize etc)
Roots and tubers (eg: potatoes, tapioca etc)
METHOD OF CONVERSION – Use intended and availability of hydrolytic agents and relative cost

Approximate chemical composition of cereal grain :–

Cereals.	Protein %	Fat %	Sol Carb. %	Crude Fiber	Mineral Matter %
WHEAT (ENG..)	10·5	2·6	78·6	2·5	1·8
MAIZE (SWEET)	12·1	9·1	74·5	2·2	2·0
SORGHUM	12·4	3·6	79·7	2·7	1·7
MILLET	13·6	5·4	77·9	1·3	1·8
RYE	13·8	1·4	79·7	2·6	2·2
BARLEY	11·8	1·8	78·1	5·3	3·1
RICE (BROWN)	11·0	2·7	83·2	1·2	1·8
OATS (WHOLE)	11·6	5·2	69·8	10·4	2·9

Chemical composition of spent spruce sulfite liquor

Component	Content % w/v
Lignosulphuric acid.	43
Hemilignin compounds.	12
Incompletely hydrolysed hemicellulose & uronic acid	7
Monosaccharides (Total)	22
D-Glucose	2.6
D-Xylose	4.6
D-Mannose.	11.0
D-Galactose	2.6
L-Arabinose	0.9
Acetic acid	6
Aldonic acid and other substrates.	10

[Forss and Passinen (1970)].

Chemical composition of wood molasses (% by weight).

Component	Percentage
Solids	52-60
Reducing sugar (as glucose)	48-50
Other carbohydrates.	0.5-1.5
Noncarbohydrates.	6-8
Ash	2-3
Nitrogen	0.065
Volatile Organic Acids	1-2

[Patel (1985) 51].

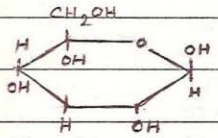
Chemical composition of rice straw :-

Component	Percentage.
Crude protein	4.5
Ether extract	1.5
Crude fiber.	35.0
lignin	4.5
Cellulose	34.0
Nitrogen free extract	42.0
Total digestable nutrients added.	43.0
Ash	16.5
Silica.	14.0
Ca	0.19
K	1.2
Mg.	0.11
P	0.10
S	0.10

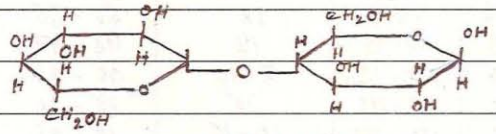
[Patel (1985), 51].

Date :

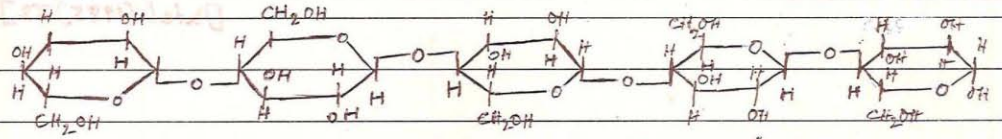
1.3.14 **Cellulosic Material :-**



β - glucose



β - Cellobiose



Cellulose chain (approx 1000 - 10,000 units)

CELLULOSIC MATERIAL

SULPHITE WASTE LIQUOR — WOOD MOLASSES — RICE STRANS

Sulphite Waste Liquor : SOURCE : Paper pulp. (Hydrolysis of wood with the help of Calcium bisulphate)
FERMENTED PRODUCTS : Industrial production of Ethyl alcohol (using Saccharomyces cerevisiae)
Growth of Torula utilis as animal feed.

Wood molasses : SOURCE : Wood cellulose (hydrolysis), saw-dust
INVOLVED MO's : Candida utilis → Pentose
Trichoderma viride → Enzyme preparation
Cellulomonas → Hydrolysis.

Rice straw and other agricultural bi-products are widely used in Asia but it is a poor quality animal feed due to lack of protein, poor palatability & bulkiness.

Chemical components of some typical commercial veg oils :-

Oil	Saponification value	Iodine value	%age saturation	Chief component acids (% w/w). Oleic acid	Linoleic acid	Linolenic acid
Olive	189-195	80-85	9-20	65-84	4-9	.....
Groundnut	189-196	85-98	18	56-65	17-26	....
Maize	188-193	117-130	12	48-47	40-42	....
Sunflower	186-194	127-136	7-10	30-35	55-65	....
Cotton seed	191-196	103-111	25	25-30	45-50	...
Linseed	189-196	170-185	10-15	15-25	15-20	45-55
Soyabeen	190-193	124-133	12-13	25-30	50-55	5-8

[Patel (1985) 52].

Analysis of corn-steep liquor samples taken :-

Components	Percentage.
Solids	40-60
Lactic acids	12-27
Total nitrogen	7.4-7.8
Amino nitrogen	2.6-3.3
Reducing sugars; as glucose	1.5-14
Ash.	18-20

[Patel (1985) 53]

Pharmamedia :-

Components	Percentage.
Protein	56
Carbohydrate	24
Oil	5
Ash [Ca, Fe, Cl, P, S]	15

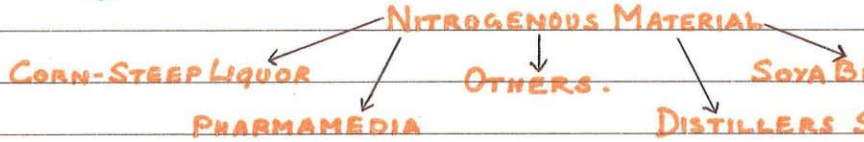
Date :

1.3.1.5 **Hydrocarbon & Vegetable oils :-**

- Less purified ones used as raw materials are relatively cheap
- Are able to produce single cell protein (SCP).
- Result in huge yeast biomass production.
- Upto 97% pure n-paraffin is achieved.

Vegetable oil: TYPES USED : Oleic acid (non drying type) olive and groundnut oil.
Linoleic acid (semi drying) maize, cotton sunflower oils.
Linolenic acid (drying) linseed, soya been

1.3.1.6 **Nitrogenous Material :-**



Corn-steep Liquor : BY PRODUCT OF : Formed during manufacture of starch gluten and other corn products and is formally know as steep water (from steeping of corns).
FERMENTED PRODUCTS : Many fungal antibiotics main penicillin.

Soyabeanmeal : BY PRODUCT OF : Formed during decilling of soyabean seed
NITROGEN : W/W 8% approximately.
PRODUCTS : Streptomycin.

Pharmamedia : PRODUCED FROM : Produced embryo of cotton seed

Date :

Distillers solubles : PRODUCED FROM : Residue of distilled alcohol (using grains or maize)
NAME : Evaporated syrup.

Others :

- Ground nut meal.
- Fish meal
- Bacto peptone
- Difco yeast extract.

1.3.2 **Pretreatment given to media :-**

- The media is thoroughly checked of its carbon, nitrogen, growth factor content.
- Precursors are added to certain medium (eg: Penicillin fermentation) which require it for better yield.
- Optimum pH is maintained for proper yield. Buffers such as $CaCO_3$ helps in pH control. Proteins are self buffers in nutrality range. Phosphates also have buffering capacity.
- Defoamers are added to media to avoid foaming (eg: Lard oil mixed with octadecanol used for penicillin fermentation.)
- The medium is made toxin free.
- Consistency of the medium is checked according to the necessity of the required fermentation.
- It is also checked of contaminants
- Availability of raw material and its composition play an important role
- Thus the media is treated accordingly and specifically for maximum yield and utility.

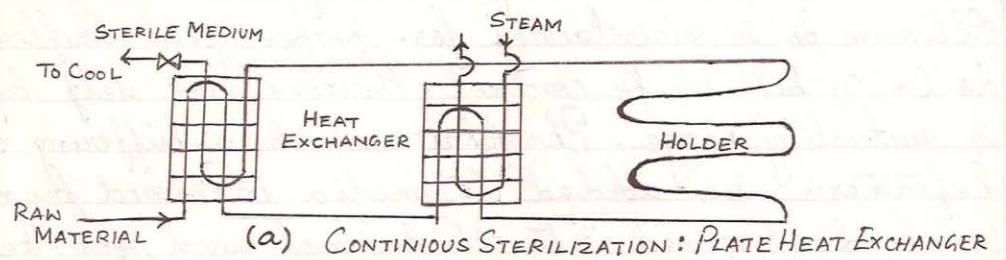
Advantage of batch cooper is that it saves time as the fermentor is unoccupied between two runs.

Limitations : i) Occupies increased plant space
ii) Higher cost of additional equipments
iii) Increased steam usage.

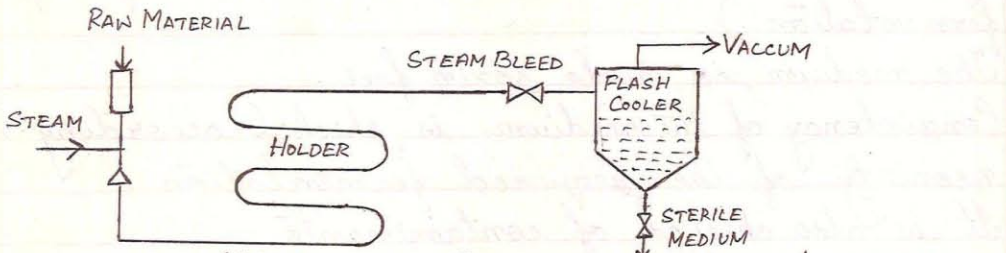
Parameters involved in continious sterilization :-

Details	Steam injection	Heat Exchanger
Holding time	1-5 minutes.	10 minutes
Holding temparature	140°c	120°c
Holding up / Cooling down	Negligible	1% contribution

Advantage :- i) Saves time & plant space
ii) Improved medium quality
iii) Economy of steam cost on application of heat exchange principle.
iv) Low sterilization temp & holding period at low pH



(a) CONTINIOUS STERILIZATION : PLATE HEAT EXCHANGER



(b) CONTINIOUS STERILIZATION : STEAM INJECTION, FLASH COOLING

Fig : 1.7

Schematic diagram for continious medium sterilization.

Date :

1.3.3 Sterilization of Production media :-

- Sterilization denotes the use of physical and chemical agents to eleminate all viable microbes from a material.
- In case of production media sterilization is decided by the chemical composition of the medium. Like in medium containing both sugare and phosphate sterilization is done seperately as they react on prolonged heati
- Sterilization is generally done by 3 methods :
  - by boiling
  - by passing live steam
  - by subjecting the medium to pressurised steam (autoclaving)
- Overcooking should be avoided for proper yield Steam sterilization may be done in two ways.
  - batchwise in fermentor
  - continious sterilization
- The temparature of the whole system is raised to 120°C and the steam is maintained inside for 20 minutes in batch cooker while in the later more flexibility of time and temparature is offered.
- Cooling is carried out by flash cooling vaccum chamber. Some medium component are not subjectable to heat and are hence sterilized by filtration (eg: Seitz filter
- Certain medium do not require sterilization at all (eg: yeast fermentation conducted at a relatively low pH)
- Thus sterilization should be conducted keeping in view the nature of the media.

TAPASYA

Page No.

Date :

1.3.4 Contamination and its control :-

- Contaminants are possible micro-organism or spoiling agents which when (accidentally) involved in fermentation process affects the same adversely and subsequently lowers the yield of fermentation products.
- They may be classified into -
  - microorganisms.
  - spoiling agents.
- Amongst the mos some are infectious in nature which would otherwise interfere with efficient fermentation processes while the others are pathogenic and devalue the fermentation processes.
  Spoiling agents also might either be inhibitory or product devaluing in nature.
- Some troublesome contaminants are —
  - Lactobacilli acts as a troublesome contaminant in Acetone-butyl alcohol fermentation using mos such as Clostridium acetobutylicum, C. butylicum & C. butyricu
  - Heavy metals inhibit or sometimes lower the rate of certain fermentation processes such as wine production.
- Contamination can be controled by :-
  - Sterilization of production media, equipment and surrounding air
  - Using antifoam reagents to stop foaming
  - Proper drawing of samples and introduction of inoculum.
  - Analysis of contaminants - yeasts, protozoa, bacteria spores and phages.

Interdependence of scale up parameters.

Scale up criterion	Designation	Small fermentor 80L		(4) Production fermentor 10,000L		
Energy input	P_o	1·0	125	3125	25	0·2
Energy input/volume	P_o/V	1·0	1·0	25	0·2	0·0016
Impeller rotation number	N	1·0	0·34	1·0	0·2	0·04
Impeller diameter	D_i	1·0	5·0	5·0	5·0	5·0
Pump rate of impeller	F	1·0	42·5	125	25	5·0
Pump rate of impeller/volume	F/V	1·0	0·34	1·0	0·2	0·04
Maximum impeller speed (max shearing rate)	N/D	1·0	1·7	5·0	1·5	0·2
Reynolds number	ND_i^2P/η	1·0	8·5	25·0	5·0	1·0

Date :

1.3.5 Scale up of fermentation :-

- The conversion of laboratory procedure to an industrial process is termed scale up.
- These conversions are generally poorly successful if applied blindly.
- Scale up is necessary only in certain cases such as :-
  - A new process is implemented in plants.
  - Mutants with 10-20% greater yield are to be introduced to large scale production as soon as possible
  - Construction of a completely new fermentation plant (rare occurrence)
- Comparison of most parameters is an important part of fermentation scaling-up.
- Most important methods of scaling-up are :-
  - Constant power consumption per unit of broth
  - Constant volumetric oxygen transfer rate.
- In geometrically similar sized reactors scaling-up is not required.

1.3.6 Buffers and Antifoaming agents :-

- For proper fermentation and maximum yield at minimal costing, optimum pH must be maintained. Buffers are components of medium that control the pH Eg : Calcium-carbonate. Proteins and amino-acids are natural buffers. Phosphates (mono & dihydrogen potassium and sodium phosphate act as good buffers.
- Certain chemicals such as lard oil mixed with octadecanol in penicillin fermentation are used to reduce foam formation. Even many mechanical methods are employed for same.

Date :

1.4 **Conclusion :-**

Much has been advocated by different authors regarding 'Fermenters & Fermentation media'. The foregoing chapter gives a brief and well formulated glimpse of all relevant topics and subtopics. The informations blended with illustrations make reading interesting and easy for others to emulate

1.5 **Glossary :-**

Ready Reference Pattern.
Script no - 3

Microbial Nutrition

Hoimee Dey
B.Sc. 1st yr (Microbiology)

Index :

'A sound beginning is ecstasy itself.....'.

THE INTRODUCTION

Nutrition: The word nutrition has been derrived from the Greek word 'nutrine' which means 'to nourish'. It is the sum of all those activities which are concerned with ingestion; digestion; absorption of the digested food into blood or lymph; oxidation of simple food to produce energy for the growth, repair, synthesis of biomolecules and egestion.

The primary necessity of all living organisms is to obtain energy and matter. Energy is required for continuation of metabolic functions. The material required for living organisms to sustain their life is called nutrient.

The raw material from which the nutrients are derrived in infinite form is known as food. Organic or inorganic substance which passes in solution through protoplasmic membrane is termed as nutrients. Inorder to encash the nutritional value of the food it must be digested into simple molecules thus making it readily absorbable through the protoplasmic membrane.

Approximate Elementary Composition of the Microbial cell.

Sl no	Elements	% of Dry Weight
1.	Carbon.	50
2.	Oxygen.	20
3.	Nitrogen.	14
4.	Hydrogen.	08
5.	Phosphorus.	03
6.	Sulphur.	01
7.	Potassium.	01
8.	Sodium.	01
9.	Calcium.	0.5
10.	Magnesium.	0.5
11.	Chlorine.	0.5
12.	Iron.	0.2
13.	All others.	~ 0.3

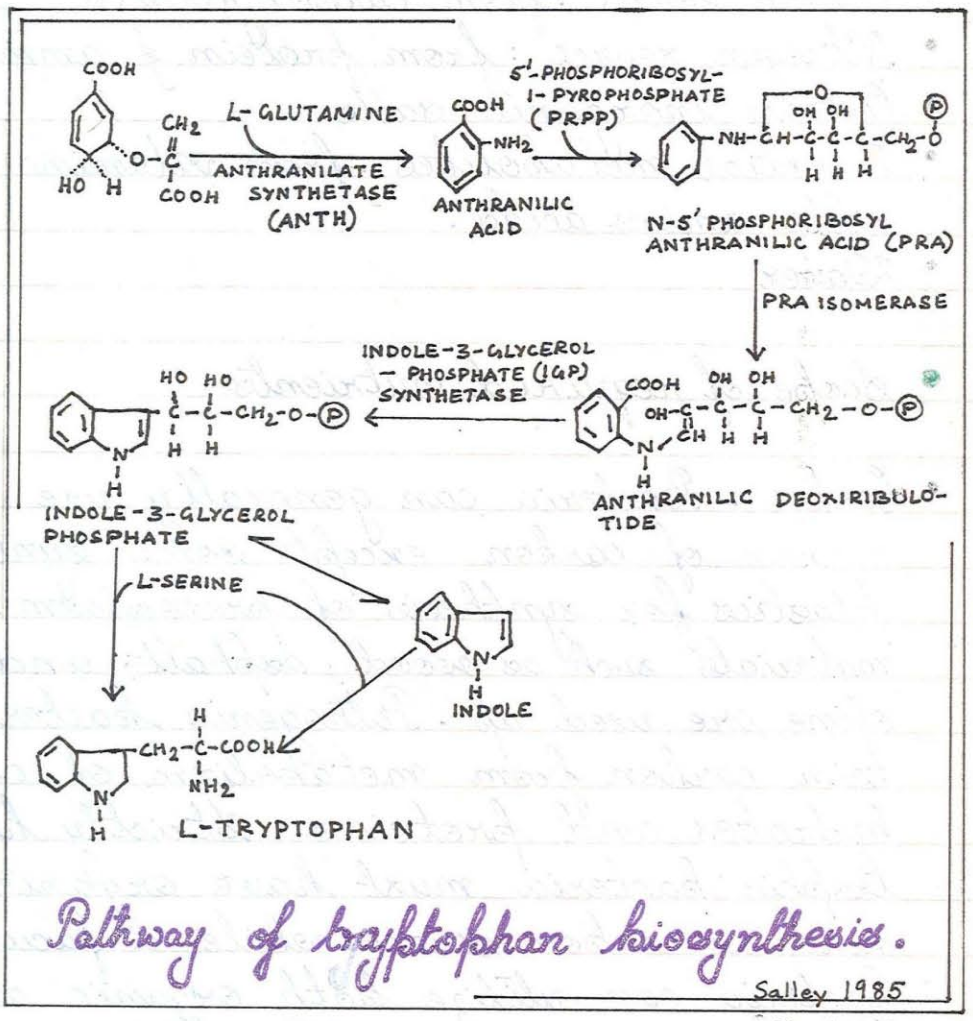
Stanier 1987

'Even the most imperceptible thing has an imparative entity.....'

- List of basic nutrional requirments
- Carbon source : from carbohydrates
- Nitrogen source : from protein & ammonia
- Certain inorganic salts
- Essential metabolites : from vitamins & possible amino acids.
- Water

- Scope of required nutrients

- Carbon : Bacteria can generally use all sources of carbon except some synthetic plastics for synthesies of protoplasm. Even materials such as wood, asphalt and gasoline are used up. Pathogenic bacteria obtain carbon from metabolism of carbohydrates and proteins. Strictly heterotrophic bacteria must have organic compound as carbon source while a facultative bacteria can utilize both organic as well as inorganic sources. Variation in carbon source in accordance with specific requirments accounts for the flora of specific milieu. Compounds other than carbohydrates used for this purpose are malic acid, succinic acid, citric acid, lactic acid & monoalcohols.



Pathway of tryptophan biosynthesis.

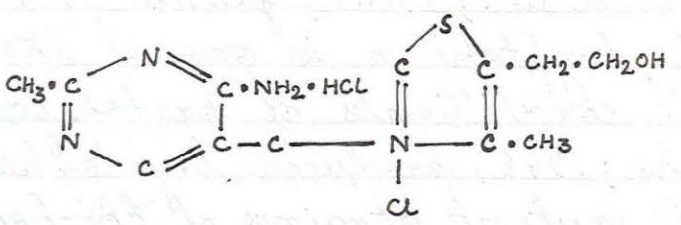
Salley 1985

The formation of lipids by bacteria is dependent upon the nature of the carbon compounds added to media.
Stephenson and Whetham ; 1922 ; reported that in a medium containing acetate, lactate and glucose, singly or in various combinations, Mycobacterium phlei synthesised sufficient lipids to become acid-fast whereas in the same medium without the carbon source the cells stain non-acid-fast.
Larson & Larson ; 1922, found that lipid synthesis occurs only if the organism uses carbon source without fermentation.
Taneda ; 1963 a, b, reported the synthesis of two straight chain fatty acids by the B subtilis when grown on nutrient medium

- Nitrogen : The strict autotrophic bacteria are able to utilize inorganic ammonium salt as the only source of nitrogen. They cannot utilize exogenous organic compounds. The strict heterotrophic bacteria do not utilize ammonium salts but must have organic nitrogen such as is present in amino acids. The facultative bacteria can exploit both the sources of nitrogen equally. Bacteria shows wide difference in their amino acid requirements.

Hunt and Pittillo; 1968, employed a chemically defined medium to determine the nitrogen requirments of a single cell of E. coli. Ammonium chloride and glucose were added to a nutritionally deficient culture of organisms. Immediately thereafter, samples were removed at 3 min interval and counts of viable cells made Appropriate calculations revcal the approximate 10^{-13} g of ammonium chloride was required per cell.
Fildes et al.; 1933, found that the amino acid, tryptophan is one of the indispensible constituents of protoplasm.
Lurcho; 1948, produced tryptophan independent mutant strains of Eberhella typhosa, capable of synthesising its own essential amino acids
Carlton; 1967, proposed a scheme for production of tryptophan in E. coli, Salmonella typimurium.

- Inorganic ions: The bacterial cells sometimes require numerous other inorganic ions. The phosphorus is used for storage of energy, sulphur containing amino acids. Some other ions such as Mg^{2+}, K^{+}, and Ca^{2+} ions act as cofactors. The other inorganic ions can be recieved from mineralized tap water itself.



Vitamin B_1 : Thiamine hydrochloride .

(Salley 1985)

Some bacteria secretes siderophores. These are substances which solubalize iron. The host iron is lightly bound to the iron transporting protein. The siderophores competes along with iron transporting protein for growth of the bacteria. The major difference between virulent and avirulent strands is the competency of siderophores. Virulent organism are more competent with the host iron due to presence of siderophores (mycobactin)

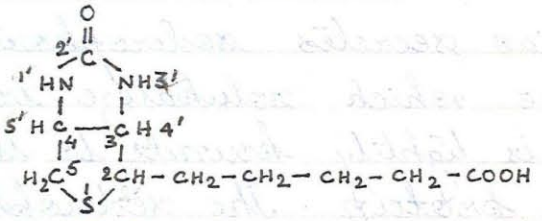
- Vitamins in bacteria as growth factor.

Growth factor or vitamins are substances which when added even in minute quantity, produce a stimulatory effect. Williams coined the term nutrilite which means the same.

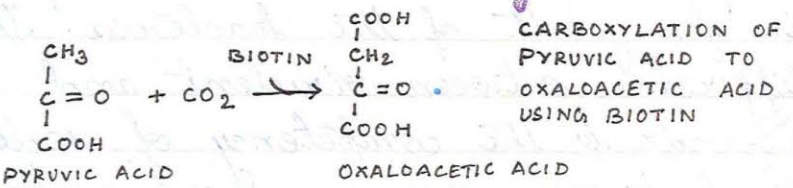
Growth of an organism in the absence of certain vitamin does not necessarily mean that the factor is not required. Rather it reveals the fact that autotrophic and facultative bacteria can synthesize at least some of the essential amino acids. The important vitamins required by bacteria are explained below :-

- Vitamin B_1 (Thiamine hydrochloride)

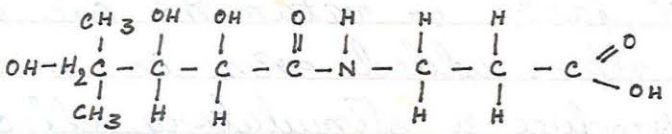
Uses :- Growth factor for all bacteria.
It serves as a precursor for co-carbo-



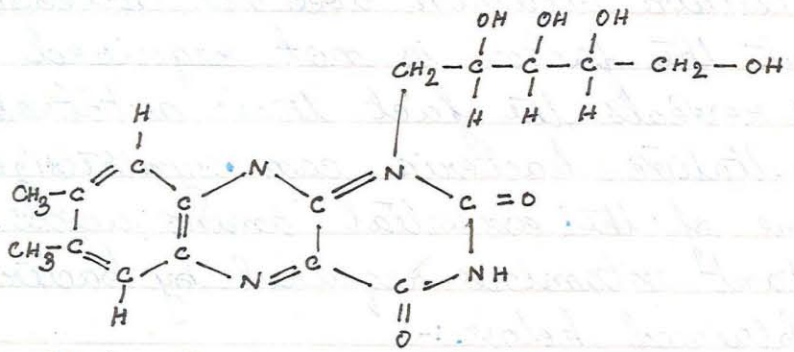
Molecular structure of Biotin.



Carboxylation using Biotin.



Molecular structure of Pantothenic acid.



Molecular structure of D-Riboflavin.

(Salley 1985)

which in turn participates in decarboxylation of α-keto acids with formation of aldehydes and carbon dioxide

- Vitamin B_7 or Vitamin H (2'keto-3-4-imidazolido-2-tetrahydrothiophene-n-Valeric acid).

Uses :- Very important part for bacterial growth A part as small as one in 50 billions can produce hundred percent increase in the yeast growth.

May sometimes participate in deamination of aspartate, threonine and serine,

Carboxylation of pyruvate, adenine and guanine,

Decarboxylation of oxaloacetate & succinate,

Oxydation of pyruvate and lactate.

It is capable of forming intramolecular hydrogen bonds.

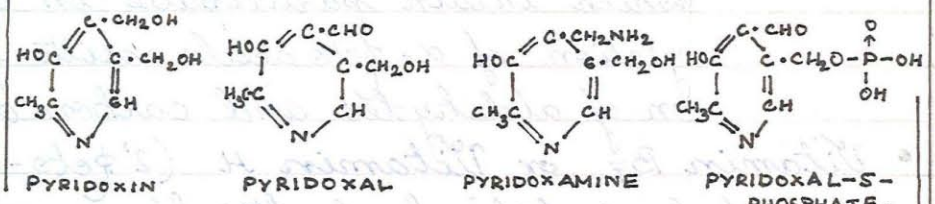
- Vitamin B_3 (Pantothenic acid)

Uses :- Acetylation of aromatic amines and cholines

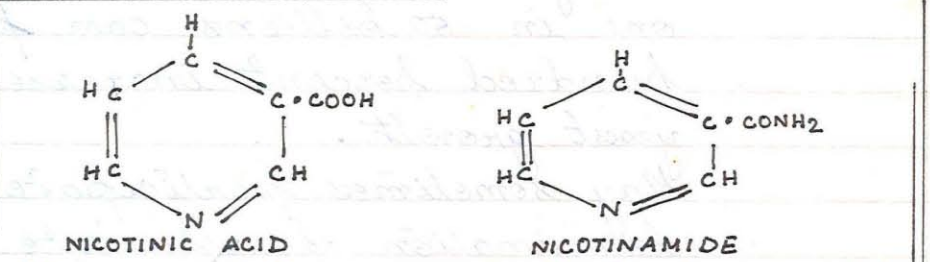
Utilization of other vitamins

- Vitamin B_2 or Riboflavin (6-7'-dimethyl-9-(D-1'-ribityl) isoalloxazine)

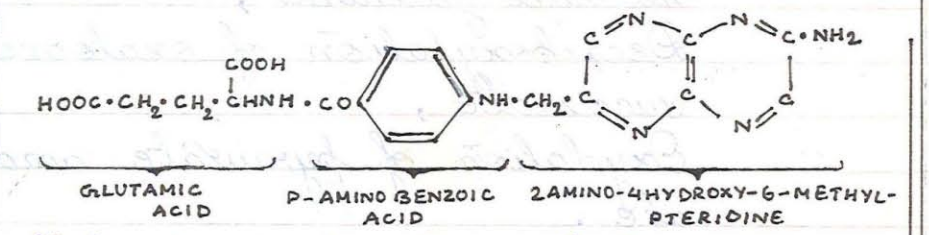
Uses :- It is a component of several en-



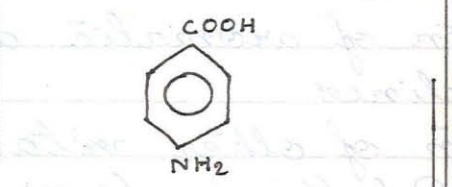
Pyridoxin & the co-occuring substances and the functionally active coenzymes.



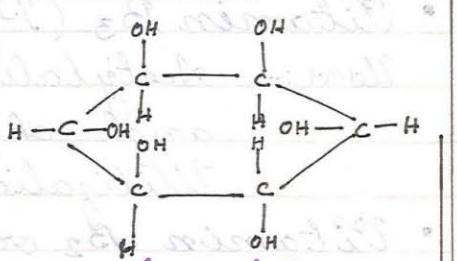
Niacin and niacinamide (molecular st..)



Molecular structure of folic acid.



pAminobenzoic acid



Inositol

(Salley 1985)

zyme known as flavoprotein such as cytochrome oxydoreductase ; lipoamide oxydoreductase etc.

- **Vitamin B_6 (Pyridoxin)**

  Uses :- It functions as transaminase for synthesis of amino acids from their keto analogs.

- **Vitamin B_5 (Nicotinic acid)**

  Uses :- Nicotinic acid and its amides are necessary for all living cells. It is a component of N.A.D and N.A.D.P

- **p-Amino benzoic acid (P.A.B.A)**

  Uses :- P.A.B.A is highly active in reversing the bacteriostatic action of sulphonamides

- **Vitamin B_9 (Folic acids)**

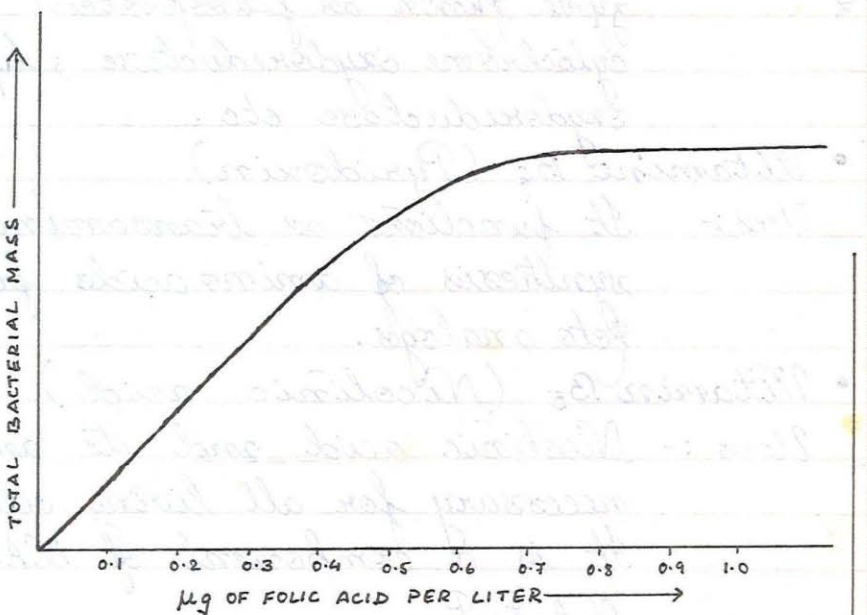
  Uses :- It synthesises certain amino acids which involve the incorporation of single carbon fragments.

- **Inositol**

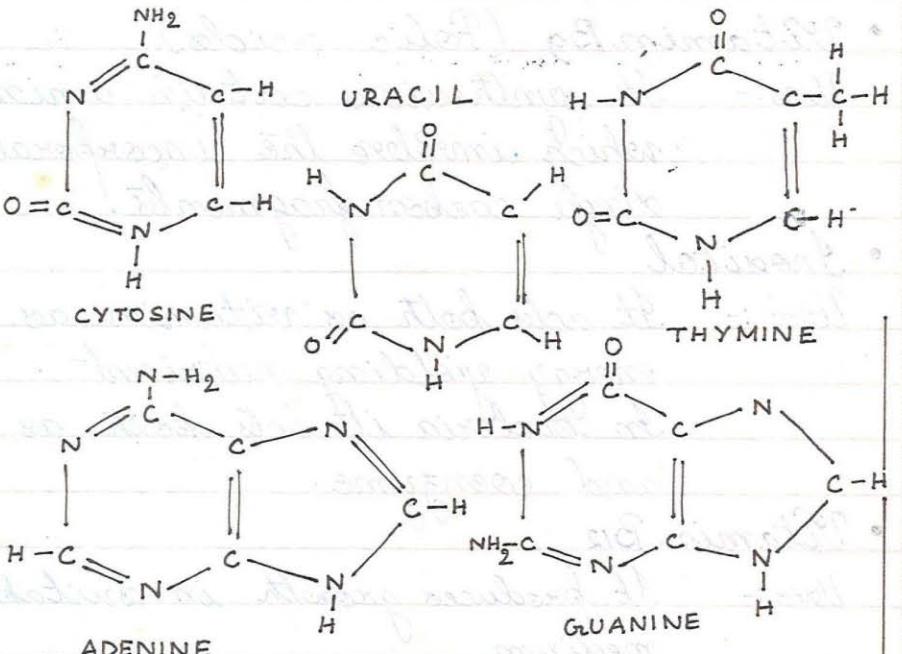
  Uses :- It acts both as vitamin as well as energy yielding nutrient. In bacteria it acts both as enzyme and coenzyme.

- **Vitamin B_{12}**

  Uses :- It produces growth in suitable culture medium. It helps in protein biosynthesis.



Graphical representation of vitamin assay by bacterium. (Volk 1984)



Common nitrogen bases.

(Salley 1985)

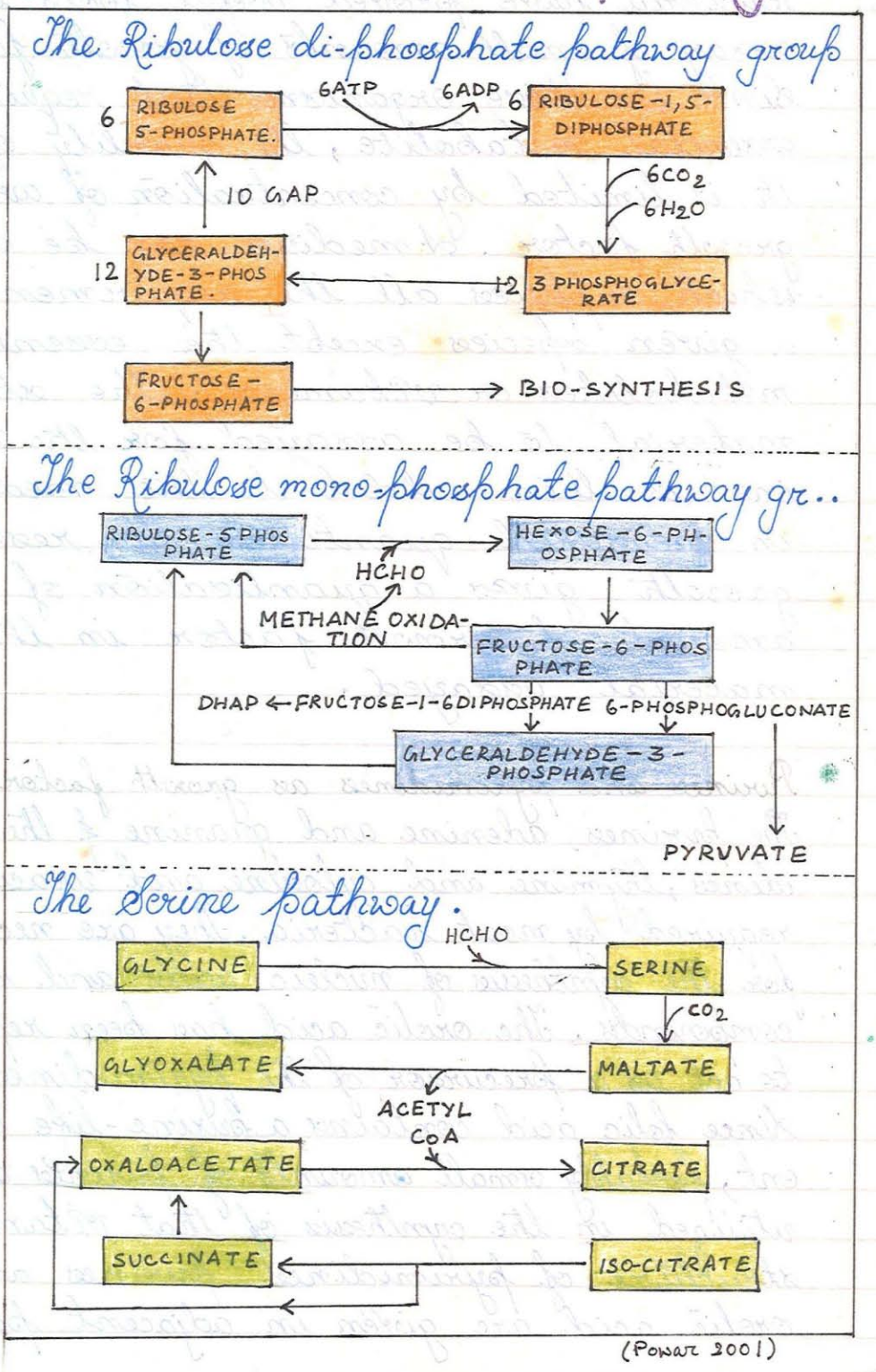
- Vitamin assay by bacterium.

Bacteria have proved useful tools in the assay of small amounts of growth factors. Since for those organisms that requir an essential metabolite, the quantity of growth is limited by concentration of available growth factor. A medium can be set up which supplies all the requirments of a given species except the essential metabolites or vitamins. The sterile material to be assayed for the vitamins is then added to the medium in measured quantities. The resulting growth gives a quantisation of the amount of growth factor in the material assayed.

- Purines and pyrimidines as growth factor.

The purines, adenine and guanine & the pyrimidines, thymine and cytosine and uracil are required by most bacteria. They are necessary for the synthesis of nucleic acids and related compounds. The orotic acid has been reported to act as a precursor of the pyrimidines. Since folic acid contains a purine-like component, probably small amounts of purines are utiliged in the synthesis of that vitamin. The structure of pyrimidines, purines and orotic acid are given in adjacent page.

The "Carbon fixation pathway."



(Powar 2001)

- Energy source

As noted the cell needs energy, capacity to do work, inorder to carry out various of its life processes, The source of energy for bacteria is generally the chemical oxidation.

The conventional difinition of autotrops includes the concept that the organisms obtain energy from oxidation of reduced inorganic chemicals. Whitenbury and Kelly, however, pointed out that autotrops do not have any common mechanism of inorganic chemical oxidation. The different substrates like : NO_2^-, NH_4^+, reduced sulphur compounds, H^+ and Fe^{2+} are oxidized by different enzyme complexes and pathways. Moreover certain organisms considered to be heterotrophs also oxidize inorganic substances. Thus Desulfovibrio and Desulfotomaculum species oxidize hydrogen and various pseudomonads oxidize thiosulphate to tetrathionate.

- Process of obtaining nutrients

- Decomposition of matter containing the nutrients.
- Absoption of simple components obtained by dissocialion of complex molecules.
- Synthesis of necessary macromolecules such as lipids, proteins, carbohydrates etc within the cell.

Classification of bacteria.

Autotrophs: CO_2 is sole source of carbon They requir only inorganic salts, CO_2 & H_2O for growth. Different energy sources are used to fix the carbon as organic compounds.

Photolithoautotrophs: Photosynthetic autotrophs. Use of inorganic electron donor.

Chemolithoautotrophs: Chemosynthetic autotrophs. Growth depends on oxidation of inorganic compound.

Chemotrophs: Organic compound as a source of CO_2. The carbon must be supplied in organic form i.e the ones formed by plant and animals.

Photoorganotrophs: These are photosynthetic heterotrophs. Use of organic electron donor.

Chemoorganotrophs: Chemosynthetic heterotrophs. Growth depends on oxidation of organic compound.

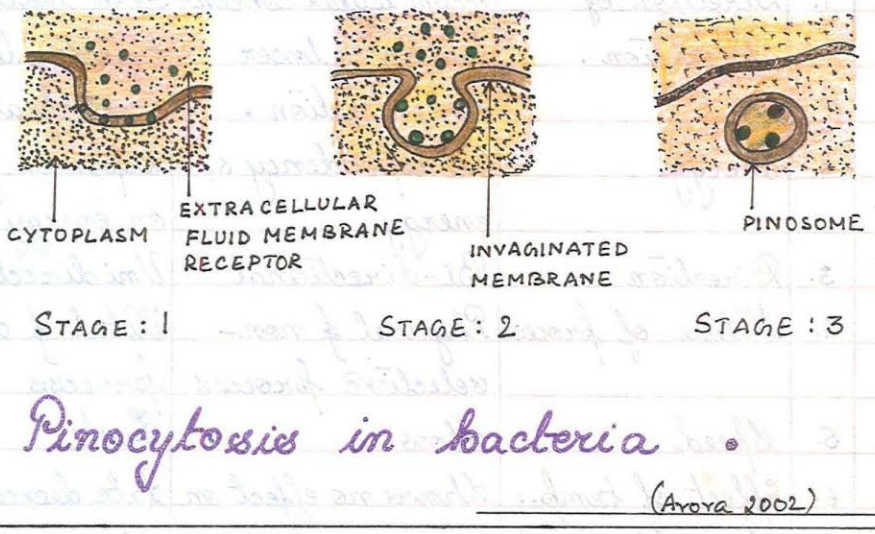
Active & Passive absorption of nutrients.

Sl.No.	CHARACTERS	PASSIVE ABSORPTION	ACTIVE ABSORPTION
1.	Direction of absorption.	From higher concentration to lower concentration.	From lower concentration to higher concentration
2.	Energy	No dependency on energy	Depends greatly on energy
3.	Direction	Bi-directional	Unidirectional
4.	Nature of process	Physical & non-selective process	Vital & selective process
5.	Speed	Slow	Fast
6.	Effect of temp.. oxygen & cyanide	Shows no effect on presence of oxygen & cyanide.	Rate decreases in absence of oxygen & presence of cyanide.
7.	Carrier protein	Not dependent	Dependent.

(Arora 2002)

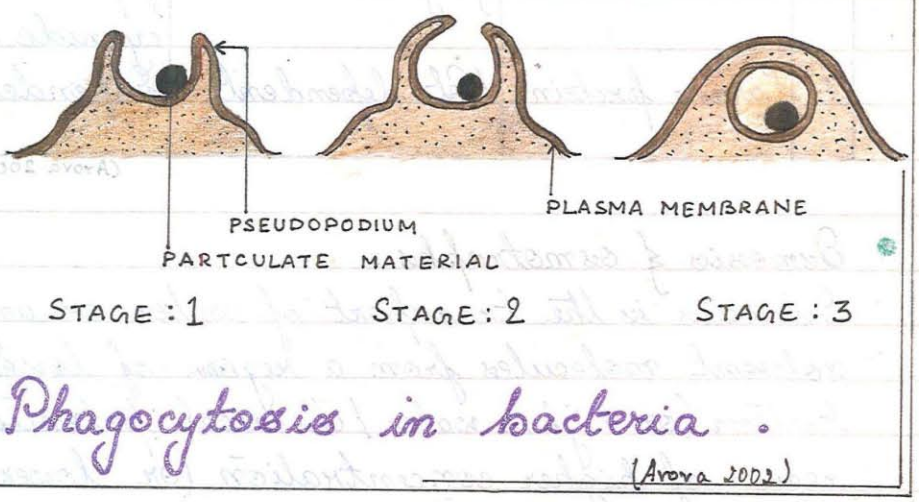
Osmosis & osmotrophs.

Osmosis is the transport of water or any other solvent molecules from a region of lower concentration (or higher water / chemical potential) to a region of higher concentration (or lower water / chemical potential) through a semi-permeable membrane. It doesnt allow movement of solute particles. The osmotrops take up all nutrients in dissolved form.



Pinocytosis in bacteria .

(Arora 2002)



Phagocytosis in bacteria .

(Arora 2002)

- **Pinocytosis**

  Gk: Pinein = to drink kytos = cell

  The process involves intake of large sized liquid nutrients. It was first shown by Lewis in 1931 A.D

- **Phagocytosis**

  Gk: Phagein = to eat

  The process involves intake of large sized solid particles including cellular-debris and microbes. It was first observed by Metchnikoff in 1883 AD

- **Syntrophism:**

  It is a type of mutualism involving the exchange of nutrients between two species. Many micro organisms synthesize vitamins and amino-acids in excess of their nutritional requirments. Others have a requirment for one or more of these nutrients. Still others synthesize certain nutrients in suboptimal amount. Hence such combination of species will grow together but not apart when the nutrition level is low.

'All's well that ends well ...'.

THE CONCLUSION

Much has been advocated by different authors and scientists to enlighten up the study of Bacterial Nutrition. The foregoing chapters give all the modernized and well formulated details of Bacterial Nutrition. The updated informations blended with picturesque illustrations make reading interesting. Any updated information or opinion by readers are requested to be placed in the notes.

'el Dorado.....'.

THE GLOSSARY

Assay: The qualitative or quantitative determination of the components of a material, such as a drug.

Oxidase: An enzyme that brings about oxidation

Pseudopodium: A temporary projection in the protoplast of amoeboid cells in which the cytoplasm flows.

Bibliography.

1. Volk, A.W & F.M, Wheeler., (1984); Microbial Nutrition In: 'Basic Microbiology' 8th Edition; J.B. Lippincott., Philadelphia, Toronto., PP 68-79.

2. Pelczar, M., Chan, E.S.C & R.N, Krieg (2001); Microbial Nutrition In: 'Microbiology' 3rd Edition; Tata Mc Graw Hills.; PP 99-105, 559.

3. Stanier, R.Y., Ingraham, J.L., Whelis, M.L & Painter, R.P., (1987); Microbial Nutrition In: 'General Microbiology' 4th Edition; Mac Millan Press Ltd., P.P 22-27.

4. Powar, C.B., H.P, Daginawala., (2001); Microbial Nutrition In: 'General Microbiology' 4th Edition; Himalaya Pub. House., P.P 421-436.

5. Arora, B.B., A.K, Sabharwal., (2002); Cell nutrition In: 'Modern's abc of Biology' Volume I., Modern Pub., P.P 91-117, 203-216.

6. Salley, A.J; (1985); Nutrition in Bateria In: 'Fundamentals of Bacteriology' 4th Edition., Tata Mc Graw Hills Pub., PP 214-245

Index:

"Well began is half done . . . ".

THE INTRODUCTION

Cultivation is the process involving the deliberate growth of microorganism on or in the nutrient media. The different microbial species growing on the same kind of media may appear differently. The knowledge of the cultural characteristics of a microbial specie is therefore useful in recognition of certain types of micro-organisms. In order to have a good cultivation of the micro-organisms it is necessary to have a good culture medium. The main aim of a culture medium is to obtain a balance in supplied nutrients to establish a well formulated colony of microbes. A good culture medium has all the necessary do's & don'ts to maximum proximity. A good and maintained medium disallows any change in internal and external experimental milieu inorder to obtain healthy microbes.

TABLE *

Medium for *Leuconostoc mesenteroides*

WATER	1 liter		
ENERGY SOURCE			
Glucose	25 g		
NITROGEN SOURCE			
NH_4Cl	3 g		
MINERALS			
KH_2PO_4	600 mg	$FeSO_4 \cdot 7H_2O$	10 mg
K_2HPO_4	600 mg	$MnSO_4 \cdot 4H_2O$	20 mg
$MgSO_4 \cdot 7H_2O$	200 mg	NaCl	10 mg
ORGANIC ACID			
Sodium acetate	20 g		
AMINO ACIDS			
DL-α-Alanine	200 mg	L-Lysine · HCl	250 mg
L-Arginine	242 mg	DL-Methionine	100 mg
L-Asparagine	400 mg	DL-Phenylalanine	100 mg
L-Aspartic acid	100 mg	L-Proline	100 mg
L-Cysteine	50 mg	DL-Serine	50 mg
L-Glutamic acid	300 mg	DL-Threonine	200 mg
Glycine	100 mg	DL-Tryptophan	40 mg
L-Histidine · HCl	62 mg	L-Tyrosine	100 mg
DL-Isoleucine	250 mg	DL-Valine	250 mg
DL-Leucine	250 mg		
PURINES AND PYRIMIDINES			
Adenine sulfate · H_2O	10 mg	Uracil	10 mg
Guanine · HCl · $2H_2O$	10 mg	Xanthine · HCl	10 mg
VITAMINS			
Thiamine · HCl	0.5 mg	Riboflavin	0.5 mg
Pyridoxine · HCl	1.0 mg	Nicotinic acid	1.0 mg
Pyridoxamine · HCl	0.3 mg	p-Aminobenzoic acid	0.1 mg
Pyridoxal · HCl	0.3 mg	Biotin	0.001 mg
Calcium pantothenate	0.5 mg	Folic acid	0.01 mg

*(Stanier 1987)

"When the going gets tough,
the tough gets going....".

- The formulation of Culture Medium.

Much of microbiology depends upon the ability to grow and maintain micro-organisms in the laboratory and this is possible only if suitable culture media are available. In addition, specialized media are essential in isolation & identification of cells, the testing of antibiotic sensitivities, water & food analysis, industrial microbiology and other activities. Even though all micro-organisms need sources of enery, carbon, nitrogen, oxygen etc, the precise composition of a satisfactory medium will depend upon the species to be cultivated. Proper selection of the micro organism with respect to the milieu enables quick and steady of micro-organisms.

- Criterion for a good culture medium.

- Adequate amount of necessary nutrients
- Addition of supplementary mineral base
- Control of pH of the medium
- Avoidance of mineral precipitation
- Control of oxygen concentration
- Avoidance of exposure to air (as molecular oxygen is inhibitory in action)
- Regular provision of carbon dioxide
- Regular provision of light.

TABLE *

Primary Environmental Factors That Determine the Outcome of Enrichment Procedures for Chemoheterotrophic Bacteria with the Use of Synthetic Media

Organic substrates, no illumination	Aerobic	Preferably nonfermentable substrate	N_2 as sole nitrogen source	(*Azotobacter* group)
			Combined nitrogen present	(Aerobes, e.g., *Pseudomonas Acinetobacter*)
	Anaerobic	Preferably nonfermentable substrate	NO_3^- as electron acceptor	(Denitrifying bacteria)
			SO_4^{2-} as electron acceptor	(Sulfate reducers)
			CO_2 as electron acceptor	(Methanogenic bacteria)
		Fermentable substrate	N_2 as sole nitrogen source	(*Clostridium pasteurianum* and related species)
			Combined nitrogen present	(Fermentative bacteria, e.g. *Enterobacter*)

*stanier (1987)

Synthetic or Defined Medium.

- Some micro-organisms particularly photolithotrophic autotrophs such as cyanobacteria and eukaryotic algae, can be grown on relatively simple media containing CO_2 as carbon source (often added as sodium carbonate or bicarbonate, nitrate or ammonia as nitrogen source, sulphur, phosphorus and a variety of minerals are added.
- Such a medium in which all components are known is called synthetic or defined medium.
- A number of chemoorganotrophic can be grown in defined medium. This type of media are widely used in research.

Complex media.

- The media that contains some ingredients of unknown chemical composition are complex media.
- Such media are very useful as a single complex medium may be sufficiently rich and complete to meet the nutritional requirements of many different microbes. In addition these are often needed because the nutritional requirements of a particular microorganism are unknown and thus a defined medium cannot be constructed. Three commonly used complex media are:
  - Nutrient broth
  - Tryptic soy broth
  - Cooked meat medium
- The medium if required is solidified using 1.5% agar.

TABLE *

Primary Environmental Factors That Determine the Outcome of Enrichment Procedures for Some Chemoautotrophic Bacteria

Absence of organic compounds in medium	Aerobic (oxygen as electron acceptor)	NH_4^+ as oxidizable substrate	(Ammonia oxidizing bacteria, e.g. *Nitrosomonas*)
		NO_2^- as oxidizable substrate	(Nitrite oxidizing bacteria, e.g. *Nitrobacter*)
		H_2 as oxidizable substrate	(Hydrogen bacteria)
		S or $S_2O_3^{2-}$ as oxidizable substrate	(*Thiobacillus*)
	Anaerobic (NO_3^- as electron acceptor)	S or $S_2O_3^{2-}$ as oxidizable substrate	(*Thiobacillus denitrificans*)
	Anaerobic (CO_2 as electron acceptor)	H_2 as oxidizable substrate	(Methanogenic bacteria)

*(Stanier 1987)

TABLE *

Primary Environmental Factors That Determine the Outcome of Enrichment Procedures for Photosynthetic Microorganisms

Light as source of energy	Absence of organic compounds	Absence of sulfide	N_2 as sole nitrogen source	(Cyanobacteria)
			Presence of combined nitrogen	(Algae)
		Presence of sulfide; anaerobic conditions	High sulfide concentration	(Green sulfur bacteria)
			Low sulfide concentration	(Purple sulfur (bacteria)
	Presence of organic compounds	Anaerobic conditions		(Purple or green nonsulfur bacteria)

*(Stanier 1987)

- General purpose media.
- Media like tryptic soy broth are known as the general purpose media as they support the growth of many bacteria.

- Enriched media.
- Some fastidious heterotrophs require special types of nutrients to support their growth. These specially fortified media are called enriched media.

- Selective media.
- Selective media favor the growth of perticular micro-organisms. The use of dyes like basic fuchsin and crystal violet favors the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria without affecting gram-negative bacteria. Endo-agar and eosin-methylene blue agar, two media widely used for the detection of
- E.coli and related bacteria in water supplies, contain dyes that suppress gram positive bacterial growth. Bacteria can also be selected by incubation with specifically utilizable nutrients. For cellulose digesting bacteria medium containing cellulose is used.
- Thus several possible selections can be made for different species of bacteria.

- Differential media.

Differential media are media that distinguish between different groups of bacteria and even

permit tentative identification of microorganisms on the basis of their biological characteristics

- Blood agar is a differential medium as well an enriched one. It distinguishes between hemolytic and non-hemolytic bacteria. Endo agar is both differential and selective. Since it contain lactose and a dye, lactose fermenting colonies appear pink to red in colour and are easily distinguished from colonies of non fermenters.

- Assay media.

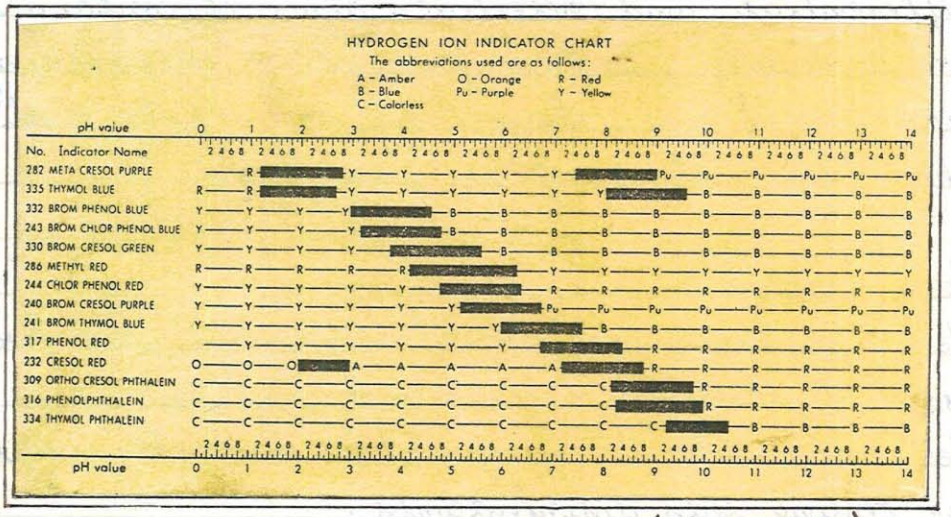
- Media of prescribed compositions are used for the assay of vitamins, amino acids and antibiotics.
- Media of a special composition are also available for testing this disinfestants.

- Media for enumeration of bacteria.

- Specific kinds of media are used for determining the bacterial content of such materials as milk and water. Their composition must adhere to prescribed specifications

- Maintenance media.

- Satisfactory maintenance of the viability and physiological characteristics of a culture over time may require a medium different from that which is optimum for growth



(Stanier 1987)

- Buffers.
- The salts of weak acids have the power of preventing pronounced changes in the reactions of solution on addition of acids and alkalies. Substances of these nature are called Buffers
- The important salts add. to nutrient media is replaced by weak basic phosphate
- A good nutrient besides being well supplied with nutrients must also be well buffed.

- pH of the medium.
- To select for acid tollerent bacteria, a low pH medium can be used.
- For example to select for lactobacilli present in cheddar cheese the pH is maintained at 5.35
- Again to select for alkali tolerent bacteria a high pH is required.
- For example to select Vibrio cholerae bacterium from stool sample 8.5 pH is required

'All's well that ends well.'

THE CONCLUSION.

Much has been advocated by different authors and scientists to enlighten the study on Bacterial Cultivation. The script has been formulated in such a fashion so as to inculcate as many relevant details as possible.

Bibliography.

1. Pelczar, M.J., Chan, E.C.S. & R.D, Krieg., (2001) Bacterial Cultivation In: 'Microbiology' Tata Mc Graw Hills; New Delhi., P.P 99-110

2. Stanier, R.J., Ingraham, J.L., Wheelis, M., & R.P, Painter., (1987). Bacterial Cultivation In: 'General Microbiology' 3rd Edition; Mac Millan Press Ltd Pub.; P.P 22-34.

3. Arora, B.B & A.K, Sabharwal., (2002)., Microbial Nutrition In: 'Modern's abc of Biology'., 4th Edition; Modern Pub.., P.P-445-462

4. Sanyal, P.D & K.C, Chatterjee., (2000)., Microbial Culture and Cultivation In: 'An Introduction to Cell Biology., Vasant Pub..., P.P 66-149, 936-945

5. Websites visited.
   - www.all the web.com.
   - www.britannica.com
   - www.eguru cool.com
   - www.goto.com
   - www.google.com

Date :

1.6 Bibliography :-

- Arora. B.B and A.K Sabharwal (2002), 'Biotechnology - fermentation', In: abc of Biology, Edn: 3rd, Publ - Modern Publishers, pp - 1226 - 1245.

- Casida L.E. Jr (1968), 'Fermentation technology', In: Industrial Microbial, Publ - John Wiley and Sons. Inc, pp - 468 - 484.

- Crueger. W and A Crueger (1982), 'Methods of Fermentation', In: Biotechnology - A Text book of Industrial Microbiology, Publ - Sinaner Associates Inc. and Science Tech Inc, pp - 58 - 96.

- Davis. B.D, Dulbecco. R, Eisen. H.N and H.S Harold (1990), 'Sterilization' In: Microbiology, Edn: 4th Publ - Harper and Row Publishers, Singapore, pp - 57 - 63.

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Page No.

- Patel. A. H (1985),
'Production Media, Industrial sterilization and Principal Types of Fermenter',
In: Industrial Microbiology, Edn: 1st
Publ - Mac Millan India Ltd,
pp - 43-86

- Powar. C. B and H. F. Daginawala
'Industrial Microbiology',
In: General microbiology, Edn: 8th
Publ - Himalaya Publishing House. pp - 90 - 108

- Prescot. S. C and C. G. Dunn (1959)
'Fermentors and Fermentation media'
In: Industrial Microbiology,
Publ - Mc Graw Hill, New York
pp - 465 - 496

- Sabarwal. R. K and S. K Sikka (1998)
'Biotechnology - Fermenters and Fementation media'
In: Advanced Biology, Edn: 5th
Publ - Modern Publishers
pp - 272 - 286

- Sanyal. G. D and P. C Chatterjee (1972)
'Advanced biotechnological processes'
In: Biology, Edn - 4th
Publ - Amar Publishers
pp - 45 - 69.

- Schlegel. H. G (1995); 'Special fermentations', In: General Microbiology, Publ - Cambridge University Press, pp - 290 - 332.
- Taylor. D. G; Green. N. P. O and G. W. Stout (1984) 'Large scale production', In: Biological Sciences, Edn: 3rd, Publ - Cambridge University Press, pp - 392 - 398.

- Periodicals:
  - Know How, The Telegraph
  - Biology Today
- Websites:
  - www.britannica.com
  - www.alltheweb.com
  - www.google.com
  - www.britannica2003.com
  - www.eguru cool.com
  - www.book.com
  - www.newbook.net

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Diseases -- Their Mini Definitions

&

Blood Grouping.

Sasoath Chakraborty.
B. Sc IIIrd Yr.
Roll- 17.

REFERENCES.

1. ; DOORLAND'S POCKET MEDICAL DICTIONARY :- : (26th Edition) Harcourt India : Saunders.

2. Talaro K.P and A. Talaro : (Third Edition : 1999) "Host defences" In "Foundation in Microbiology" (PP 392-395). WCB-Mc Graw Hill (Toronto).

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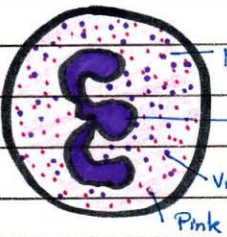
ADDITIONAL INFORMATION

I. Blood Staining :-

Diseases & Their Mini Definitions.



Neutrophil.

Increase in number of neutrophils ⟹ ① SEPTIC ENDOCARDITIS ② PUS FORMATION

• SEPTIC ENDOCARDITIS → (-kahr'-di-tis)

→ exudative and proliferative inflammatory alteration of the endocardium,

→ Usually characterized by the presence of vegetations on the surface of the endocardium (within the heart) or in the endocardium itself. and most commonly involving heart valve, but also affecting the inner lining of the cardiac chambers or the endocardium elsewhere.

→ **Causal organisms :-** Streptococci, Staphylococci, Enterococci Gonococci & Gram negative bacilli.

• PUS FORMATION :→

→ a protein rich liquid inflammation product made up of cells. (leukocytes) a thin fluid (liquour puris) & cellular debri.

→ **Causal organism:-** Strept pyogenes, Staph aureus etc.

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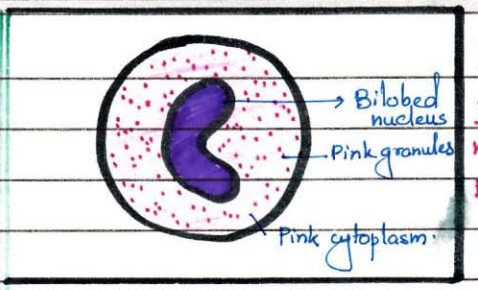


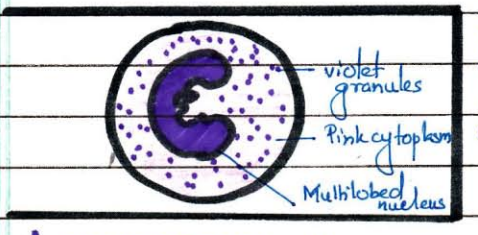
Eosinophils

Increase in number of Eosinophils ⟹ SCARLET FEVER.

- **SCARLET FEVER :→**

→ An acute disease caused by Group A β-hemolytic streptococci, marked by pharyngotonsillitis and a skin rash caused by an erythrogenic toxin produced by the organism

→ The rash is a diffuse, bright red erythema and desquamation of the skin begins as fine scaling with eventual peeling of the palms and soles.

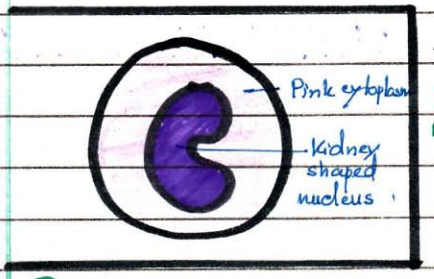


Basophils.

Increase in no of Basophils → BASOPHILIA
→ VIRAL INFECTION
→ LEUKOPENIA.

- **LEUKOPENIA. :→.**

Reduction in the number of leukocytes in the blood below about 5000 per cubic m.m. Basophilic Leukopenia pertains to Basophilia



Monocytes.

Increase in no of Monocytes → RICKETSIAL DISEASE
→ ROCKY MOUNTAIN SPOTTED FEVER.

- **RICKETSIAL DISEASE :-** Caused by Rickettsia.

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ROCKY MOUNTAIN SPOTTED FEVER

→ Infection with Rickettsia rickettsii

→ Transmitted by ticks, marked by fever, muscle pain, & weakness followed by a macular petechial (red spot due to escape of a small amount of blood) eruption that begins on the hands & feet & spreads to the trunk and face with other symptoms in the CNS & elsewhere.

— x —

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BLOOD GROUPING

THE BASIS OF HUMAN ABO ISOANTIGENS AND BLOOD TYPES.

→ The existence of human blood types was first demonstrated by an Austrian pathologist, **Karl Landsteiner in 1904**

→ While studying incompatibilities in blood transfusions, he found that the serum of one person could clump the red blood cells of another.

→ Landsteiner identified four distinct types, subsequently called the **ABO** blood groups.

→ Like the MHC antigens on White Blood cells, the ABO isoantigen markers on red blood cells are genetically determined & composed of glycoproteins.

→ These ABO antigens are inherited as two (one from each parent) of three alternative alleles * **A, B or O.**

→ **A & B** alleles are dominant over **O** and codominant with one another.

→ This mode of inheritence gives rise to four blood types (phenotypes), depending on the particular combination of genes.

→ Thus a person with an **AA or AO** genotype has **type A** blood; genotype **BB or BO** gives **type B**; genotype **AB** produces **type AB**; and genotype **OO** produces **Type O.**

IMPORTANT POINTS ABOUT BLOOD TYPES.

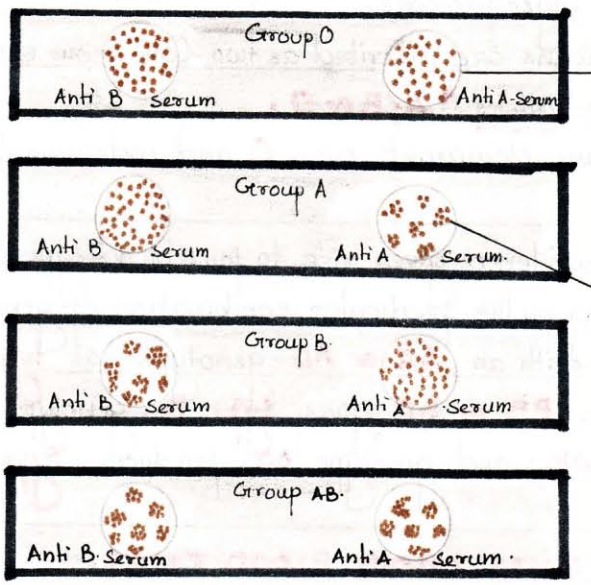
(1) They are named for the dominant antigen(s)

(2) The RBC's of type O persons have antigens, but not A & B antigens.

TABLE-1 CHARACTERISTICS OF ABO BLOOD GROUPS.

Genotype	Phenotype A & B* RBC Antigen	Prevalence in Population**	Serum Content of Antibodies.
OO	Neither	Most common	Both anti a & anti-b.
AA, AO	A	Second most common	Anti-b.
BB, BO	B	Third most common	Anti-a
AB	AB	Least common	Neither antibody

Legend: * Capital letters generally denote antigen; lowercase denotes antibody.
** True of most large population of mixed racial & ethnic groups.



Legend: Red blood cells (erythrocytes) of the type indicated at the top of each slide are mixed with blood serum of the type indicated below each reaction mixture (circle)

→ A clumped pattern of cells within a circle indicates that agglutination occurs.

FIG 1. AGGLUTINATION REACTIONS CONTROLLED BY THE ABO BLOOD TYPE LOCUS IN HUMANS :-

(3) Tissues other than RBC's carry A & B antigens.

GENETIC BASIS :- ABO BLOOD TYPE ALLELES IN HUMANS :-

Table 2 :- Genotypes & the corresponding Phenotypes (Blood Gr. Types) for the ABO Locus (Human)

GENOTYPE	PHENOTYPE.
$I^A I^A$ a $I^A I^O$	A
$I^B I^B$ a $I^B I^O$	B
$I^A I^B$	AB
$I^O I^O$	O

→ One of the most firmly established series of multiple alleles in humans involves the genetic locus controlling the blood types **A, B, AB & O.**

→ The ABO locus has three common alleles **I^A, I^B, & I^O**

→ I^A & I^B are **codominant** ($I^A I^B$ heterozygotes have both A & B antigens on their RBC's) & I^O is **recessive** ($I^O I^O$ homozygotes have no ABO antigens on their RBC's; $I^A I^O$ & $I^B I^O$ heterozygotes have A & B antigens, respectively, on their RBC's.

→ The ABO locus controls the type of glycolipids found on the surface of erythrocytes, apparently by specifying the type of **glycosyl transferases** (enzymes catalyzing the synthesis of polysaccharides) synthesized in the RBC's.

→ The specific types of glycolipids on the red cell surface provide the antigenic determinants that react with specific antibodies present in the blood serum.

→ Humans, like all other mammals, produce antibodies & circulate them in the blood serum as a defence mechanism against foreign substances.

Fortunately, no antibodies are synthesized (in normal individuals)

TABLE:- 3.

BLOOD TRANSFUSION COMPATIBILITIES FOR THE ABO BLOOD GROUPS.

BLOOD GROUP	TERMINAL SUGARS OF AGS PRESENT	ANTIBODIES PRESENT	RED CELL TYPES AGGLUTINATED	TRANSFUSIONS ACCEPTED FROM.
A	A (galactosamine)	Anti- B	B, AB	A or O
B	B (galactose)	Anti- A	A, AB	B or O
AB	A (galactosamine)	None	None	A, B, AB or O
O	None	Anti-A & Anti-B	A, B and AB	O.

Fig: 2.

INTERPRETATION OF BLOOD TYPING.

Legend : In this test, a drop of blood is mixed with a specially prepared antiserum known to contain antibodies against the A, B & RH antigens.

Fig ⇒ If that particular ag is not present, the R.B.C's in that droplet do not agglutinate & form an even suspension.

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that react with antigens present on the individual's own cells. However, when type A blood & type B blood are mixed, the anti A antibodies in the type B blood serum react with the antigens on the type A blood cells, & vice versa, which produces agglutination a clumping of cells [fig (v)]

→ Cross-matching blood types to determine compatibility is thus essential in blood transfusions.

→ In this process, blood donors and recepients are tested for the presence of antigens & antibodies that are incompatible.

→ Table (iii) summarizes the cell surface antigenic determinants & the serum antibodies present in the four major ABO blood types.

TRANSFUSIONS. → Individuals with blood type AB have both A & B antigens on their erythrocytes, but no anti A & B antibodies in their blood serum.

→ Type O individuals lack both ags, but carry both anti-A & anti-B abs in their blood serum

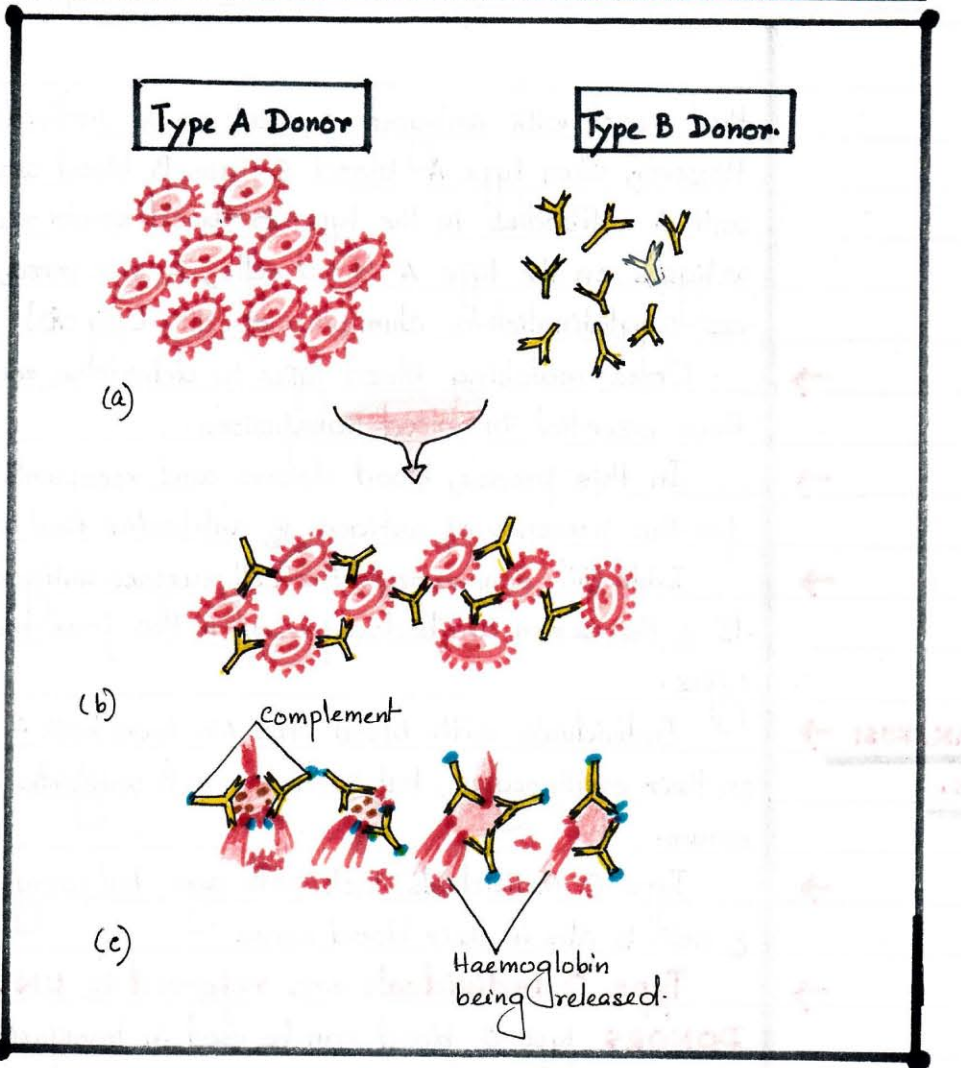
→ **Type O individuals are referred as UNIVERSAL DONORS**, type O blood can be used in transfusion for individuals of any blood type if the blood is introduced slowly enough to permit sufficient dilution of the Anti-A & Anti-B abs present in the serum of the donor.

→ Type AB persons are consequently called **UNIVERSAL RECEPIENTS.**

DEGREES OF ADVERSE REACTIONS IN TRANSFUSIONS.

Transfusion of the wrong blood type causes various degrees of adverse reaction.

Fig: 3 : MICROSCOPIC VIEW OF A TRANSFUSION REACTION



Legend :- (a) Incompatible blood. The red blood cells of the type A donor contain ag A, while the serum of the type B recipient contains anti-A abs that can agglutinate donor cells.

(b) Agglutination particles can block the circulation in vital organs.

(c) Activation of the complement by ab on the RBC's can cause haemolysis & anaemia. This sort of incorrect transfusion is very rare because of the great care taken by blood banks to ensure a correct match.

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→ The severest reaction is massive hemolysis when the donated red blood cells react with recipient antibody & trigger the com-plement cascade (fig. 3).

→ The resultant destruction of red cells leads to systemic shock & kidney failure brought on by the blockage of glomeruli (blood filtering apparatus) by cell debris.

→ Death is a common outcome.

Other reactions caused by RBC destruction are **fever, anemia & jaundice**

→ A transfusion reaction is managed by immediately halting the transfusion, administering drugs to remove hemoglobin from the blood, and beginning another transfusion with RBC's of the correct type.

A CLOSER APPROACH.
MICROFILE: THE ORIGIN OF ABO ANTIGENS.

→ **The A and B genes** each code for an enzyme that adds a terminal carbohydrate to RBC receptors during maturation. **RBC's of Type A** contains an enzyme that adds **N-acetyl glucosamine** to the receptor; RBC's of **type B** have an enzyme that adds **D-galactose**; RBC's of **type AB** contain both enzymes that add both carbohydrates; and RBC's of **type O** lack the genes & enzymes to add a terminal molecule.

→ The genetics of ABO ags were once used to rule out paternity. For eg if a man is type A, the mother type O, & the child type B, we know this man could not have fathered this child.

→ However this same logic cannot prove paternity. If the child is type A instead, it is this same logic for the man to be the

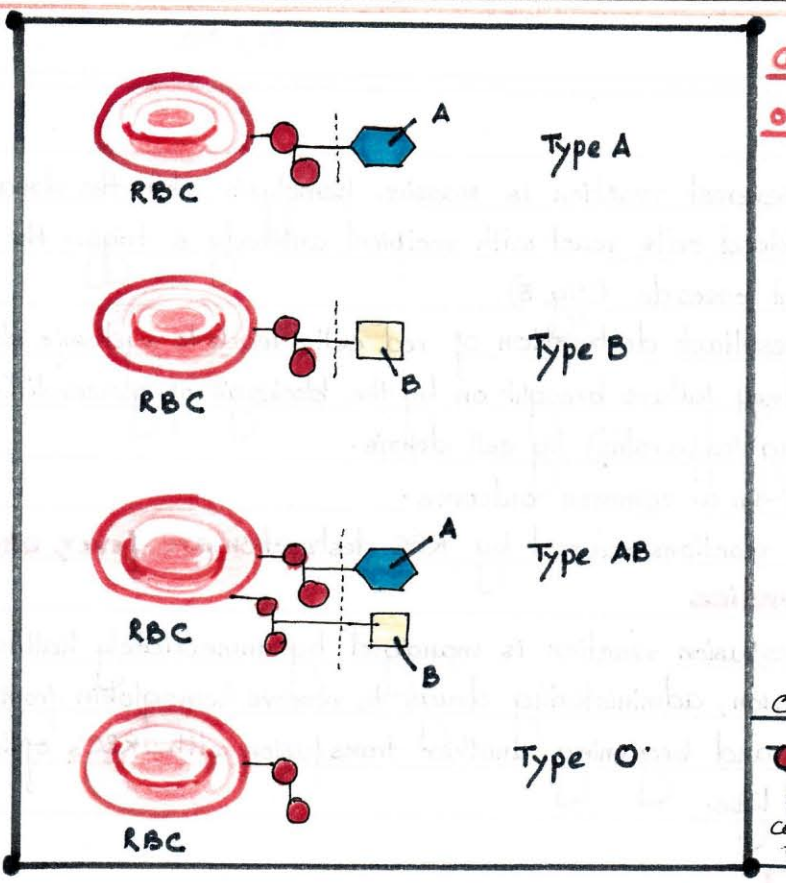
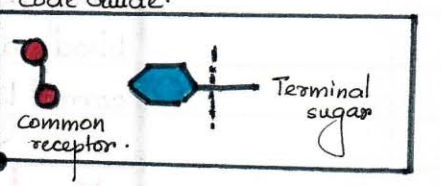


Fig 4:-
GENETIC BASIS FOR AB AGS. ON RBC.



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father, but so could some other man with blood type A.

→ Highly sensitive methods based on specific & variable MHC ags & DNA fingerprinting have been developed to gather more precise evidence of paternity & maternity (in cases of kidnapping & adoption, for instance).

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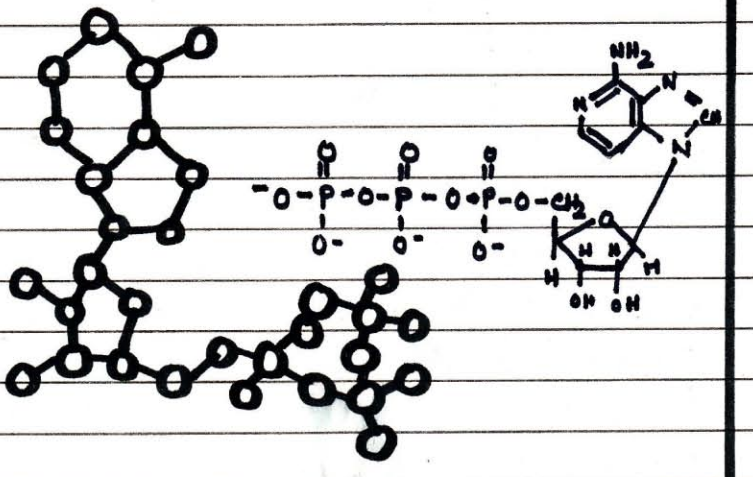
METABOLISM

ATP

(•) Name : Saswati Chakraborty
(•) Std : B.Sc III Yr
(•) Roll : 17



Adenosine Tri Phosphate.

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REFERENCES :-

1. A.L. Lehninger : (1995) "Bioenergetic Principles and the ATP cycle", Metabolic and Energy Transfer Pathways : A Survey", "Electron transport and oxidative Phosphorylation" IN FUNDAMENTALS OF BIOCHEMISTRY (PPs - 267 - 289 - 813, 865 - 895) PUB: SPARKS (MARYLAND).

2. Martin. W.D., Mayes A.P., and V.W Rodwell : (1985) "Bioenergetics" In Harper's Review of Biochemistry (PPs 64 - 69) PUB: MARUZEN ASIAN EDITION, Lange Medical Publications

3. Champe. P.C and R.A. Harvey : (1988) "ATP AS ENERGY CARRIER" IN LIPPINCOTT'S ILLUSTRATES REVIEWS : BIOCHEMISTRY (PPs : 99-105) Pub: (7th edition).

4. Todd. W.R., West E.S and H.S Mason (1985) "Introduction to Intermediary Metabolism" In TEXTBOOK OF BIOCHEMISTRY (PPs 850-851, 884-895)

5. Mahler. H.R and E.H. cordes : (1974) Aerobic Respiration IN BIOLOGICAL CHEMISTRY (2nd ed^{n}) (PP- 378-382) PUB: Harper and Row Publishers (New York) - [HARPER INTERNATIONAL]. - [New York]

6. Conn. E.E and P.K stump : (1974: 4th ed^{n}) Biological Energetics In OUTLINES OF BIOCHEMISTRY (PP- 142-148) Pub: WILEY EASTERN LTD (California).

7. D. E. Sadave : (1992). Energy Transduction : Mitochondria, In CELL BIOLOGY (organelle-structure function) (PPs - 110 - 114)
Pub : Jones and Bartlett Publishers (California).

8. PELCZAR . J.M., CHAN. E.C.S and N.R.KRIEG : (1993) Microbial Metabolism In MICROBIOLOGY (PP - 171-196)
Pub : Tata Mc Graw Hill Publishing Co Ltd. (Delhi).

9. A. J. SALLE : (1992) Fermentation of Carbohydrates In FUNDAMENTAL PRINCIPLES OF BACTERIOLOGY (PP - 475-505)
Pub : Tata Mc Graw Hill Publishing Co Ltd (Delhi).

10. Power. C.B and H.F Daginawala : (1991) Carbohydrate Metabolism In GENERAL MICROBIOLOGY. (PP - 488)
Pub : Himalayan Publishers (Mumbai).

11. Duodoroff Michael : (7th Edition) ATP, Metabolism . In ENCYCLOPEDIA OF SCINCE AND TECHNOLOGY (Vol I and II) (PP 120 - 121, 4 - 5)
Pub : Tata Mc Graw Hill Publishing Co Ltd (Delhi)

12. C. J. Avers : (1992) Aerobic Respiration In MOLECULAR CELL BIOLOGY. (PP - 328 - 329).
Pub :

13. L. STRYER : (1987) "ATP" In BIOCHEMISTRY (3rd edition)
Pub : H. Freeman and company (PP. 950, 953, 415, 416).

14. H. G. Schlegel : (1995) Modes of Energy Yielding Metabolism In "GENERAL MICROBIOLOGY" (PP : 343). Pub : Cambridge University Press.

15. Website : www.google.com.

16. Devlin. R. M and F. H. Witham : (1997) Energy Liberation and Utilization In. PLANT PHYSIOLOGY. (PP-318 - 319). Pub: CBS Publishers & Distributors (Delhi).

17. Dubey. R. C and D. K Maheshawari : (2000) ATP In GENERAL MICROBIOLOGY (2nd Ed") (PP-267 - 269) Pub : S. Chand and company Ltd.

18. Roleries., E. D. P and B. M. F De. Roberts Jr : (1997) ATP IN CELL AND MOLECULAR BIOLOGY (PP-26) Pub : B. I. Waverly Co Ltd.

19. M. Swaminathan : (1981) ATP in BIOCHEMISTRY OF STUDENT (P.P- 461-532) Pub : Geeta Book House (Mysore).

20. D. Friefelder : [2nd edition 1994] ATP IN MOLECULAR BIOLOGY (PP - 30-36) Pub : Narasa Publications House.

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METABOLISM

AN OVERVIEW

- Definitions of metabolism.
- Catabolism and Anabolism.
- Classification of micro-organisms on the basis of energy & carbon sources.
- Bioenergetics.
- Coupling through ATP and through pyridine nucleotides.

INTRODUCTION : [Definition].

— The term metabolism denotes all the organized chemical activities performed by a cell, which comprise two general types :

→ energy production

→ energy utilization

— The term intermediary metabolism is a rather incomplete definition which only highlights by eliciting as the sum total of all the enzyma-tic reactions occurring in the cell.

— Four specific functions of metabolism are :-

→ (1) To extract chemical energy from the environment, either from organic nutrients or from sunlight.

→ (2) To convert exogenous nutrients into the building blocks or precursors of the macromolecular components of cells.

→ (3) To assemble the building blocks into proteins, nucleic acids, lipids.

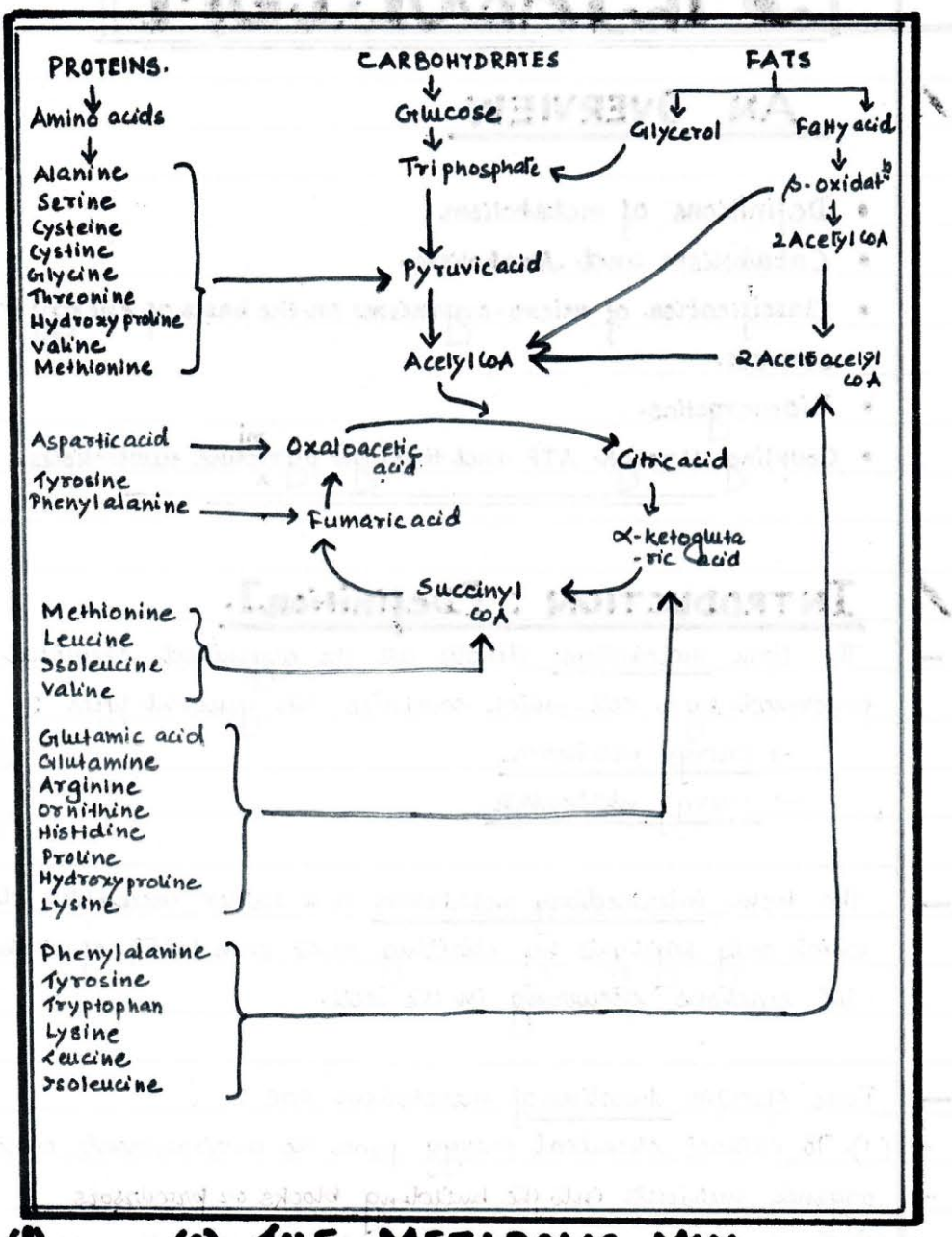

Fig: 4) (*) THE METABOLIC MILL

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and other characteristic cell components and

→ (4) To form and degrade those biomolecules required in specialized functions of cells.

TYPES OF METABOLIC REACTIONS.

METABOLISM

CATABOLISM.

(•) It is the enzymatic degradation, largely by oxidative reactions, of relatively large nutrient molecules (carbohydrates, lipids & proteins) coming either from the environment of the cell or from its own nutrient storage depots into a series of smaller, simpler molecules e.g lactic acid, acetic acid, CO_2, ammonia a, urea.

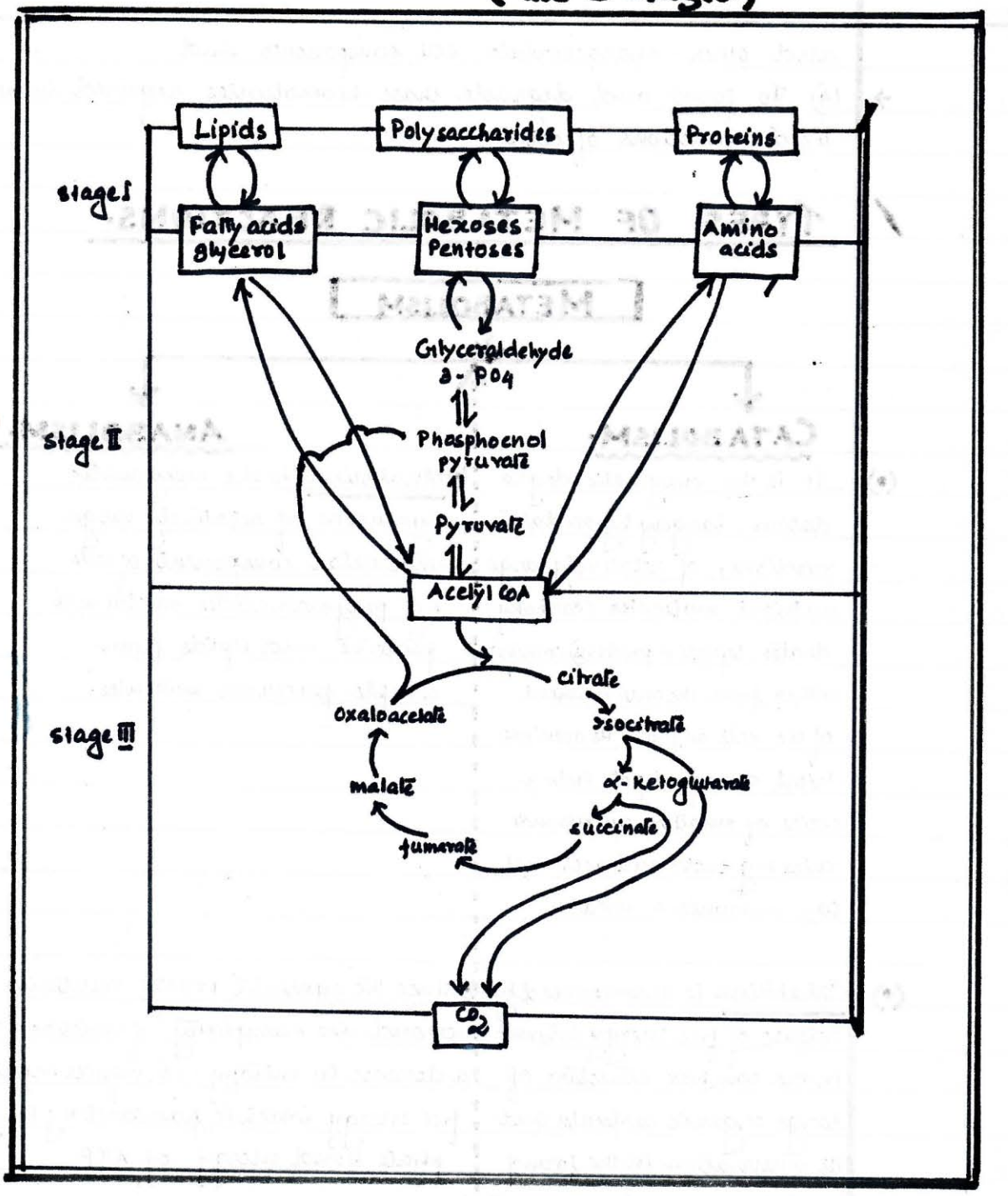
(•) Catabolism is accompanied by release of free energy inherent in the complex structure of large organic molecules and its conservation in the form of the phosphate bond energy of ATP.

ANABOLISM.

(•) Anabolism is the enzymatic synthesis of relatively large molecular components of cells e.g polysaccharides, nucleic acids proteins and lipids from simple precursor molecules.

(•) Since the synthetic process results in increased size & complexity of structure & thus a decrease in entropy, it requires input of free energy which is furnished by the phosphate bond energy of ATP.

Fig2: (*) CATABOLISM, ANABOLISM, AMPHIBOLISM
(The 3 stages.)



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AMPHIBOLIC PATHWAY :-

Although the pathways of catabolism and anabolism are not identical the stage III [fig 2] constitutes a central meeting ground or pathway which is accessible to both. This central route, is called an amphibolic pathway. (amphi → dual).

CLASSIFICATION OF M.O.S ON THE BASIS OF ENERGY CARBON SOURCES :

•> Division on the basis of utilization of carbon source.

- AUTOTROPHS
  - Autos : self
  - trophos : feeders
  - → Photo autotrophs
  - → Chemolithotrophs
- Heterotrophs
  - Hetero : other
  - trophos : feeders

•> Division on the basis of energy source

- Phototrophs
  - Photo organo trophs
  - Photolitho trops.
- Chemotrophs
  - Chemoorgano trophs
  - Chemo lithotrophs.

•> Division on the basis of oxidizing agent for nutrient breakdown.

- (Aerobes) [those which grow in presence of O_2]
- (Anaerobes) [those which grow in absence of O_2]

Fig 3 (*) The increase of entropy :

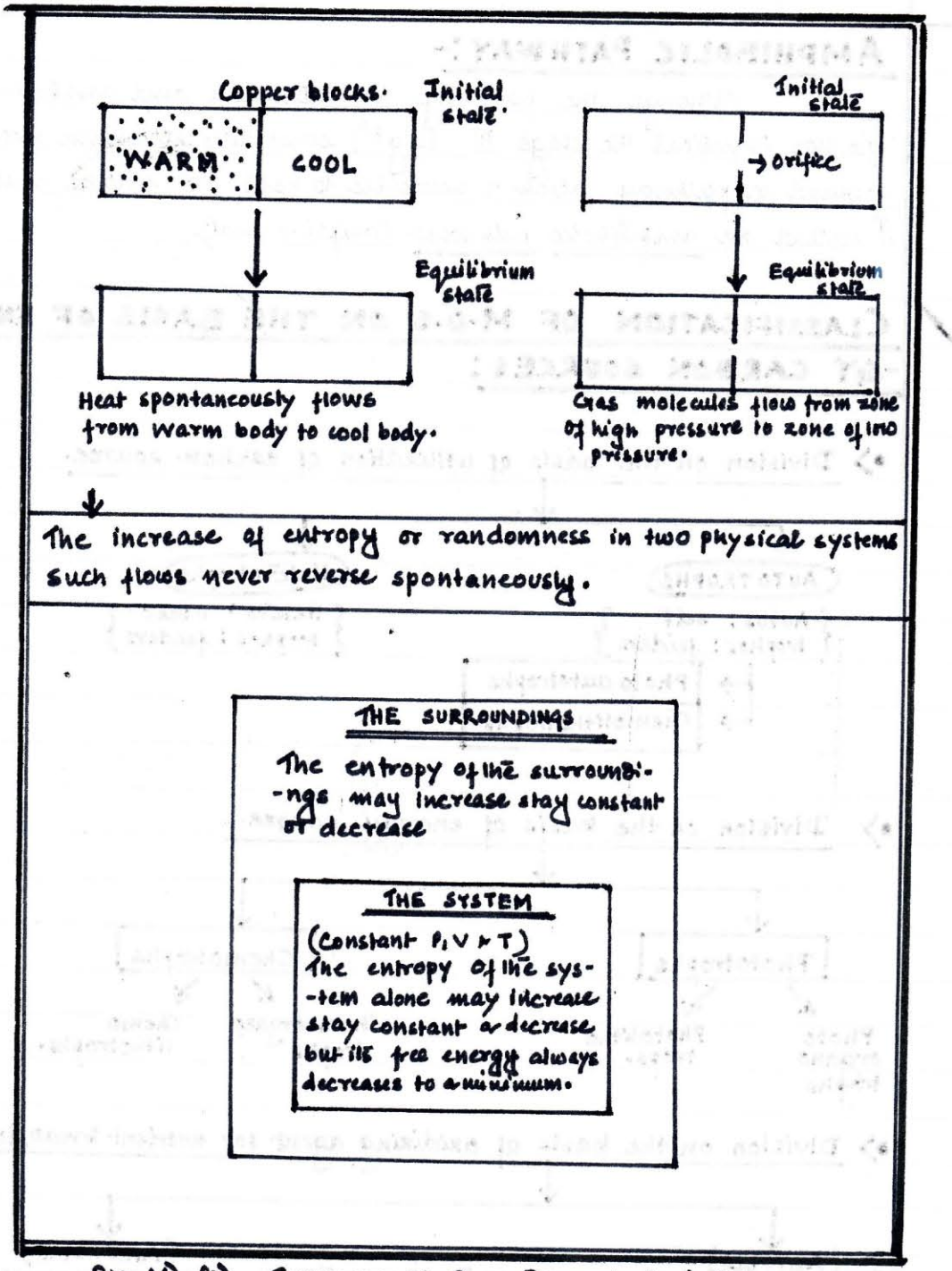

Fig (4) (*) Summary Of Free Energy And Entropy.

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BIOENERGETICS:

(TERMINOLOGIES INVOLVED).

(•) **ENTROPY** :- Entropy is defined (for the moment) as the degree of disorder or randomness. ['S']

(•) **EQUILIBRIUM** :- An Equilibrium is defined as a state in which no further net chemical or physical change is taking place and in which temperature, pressure and concentration are uniform throughout the system.

∴ All "real" processes occurring in our physical world including the process of life are Irreversible ∴

(•) **FREE ENERGY** :- Entropy changes during chemical reactions are not always easily measured or calculated. However the change in entropy during a process is quantitatively related to changes in total energy of the system by a third function called the Free Energy. [ΔG]

(•) **ENTHALPY** :- The change in function is known as enthalpy

(•) **IMPORTANT EQUATIONS**:

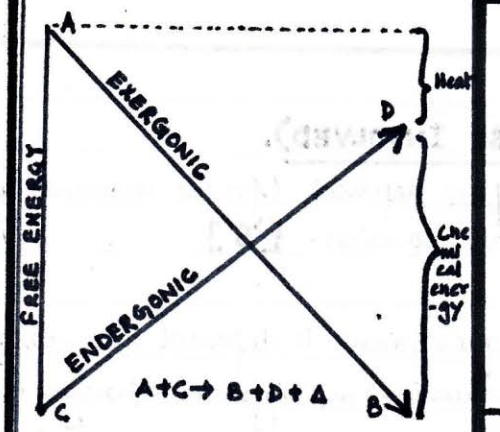
(1) $\Delta G = \Delta H - T\Delta S$

(2) $\Delta H = \Delta E + \Delta PV$

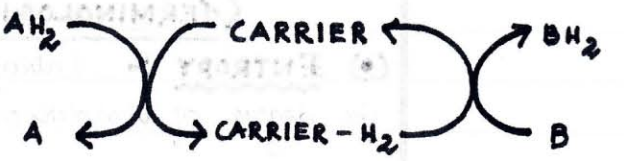
(3) $\Delta G = \Delta E + T\Delta S$

(4) $\Delta E = \Delta G + T\Delta S$

(•) "COUPLING - REACTION"



Coupling of an exergonic to an endergonic reaction. → Fig (6)(•)



Coupling of dehydrogenation and hydrogenation reactions by an intermediate carrier → Fig (6)(•)

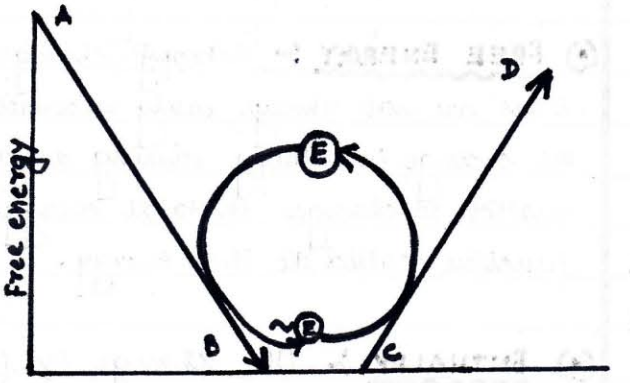
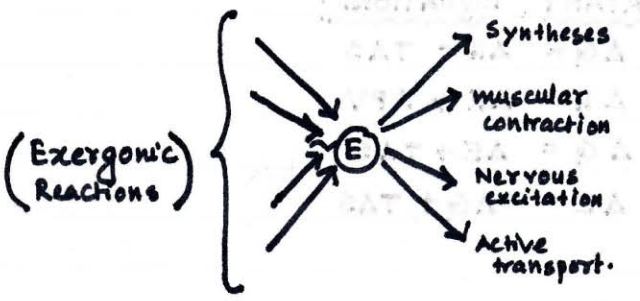


Fig (7) → Transference of free energy from an exergonic to an endergonic reaction through the formation of a high energy intermediate compound. (•)



(•) Fig (8) → Transduction of energy through a common high-energy compound to energy-requiring (endergonic) biologic processes.

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BIOENERGETICS OF COUPLED REACTION:-

Coupling – ATP & Pyridine Nucleotides.

→ First reaction in the glycolytic pathway.

[The phosphorylation of glucose to glucose 6-P which is highly endergonic and would not proceed as such under physiologic conditions]

(1) Glucose + P_i ⟶ Glucose 6-P + H_2O

($\Delta G^{0'} = +13.8$ KJ/mol)

(2) ATP ⟶ ADP + Pi ($\Delta G^{0} = -36.8$ KJ/mol)

Reaction couples with another reaction (hydrolysis of the terminal PO_4 of ATP) that is more exergonic.

(3) Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose 6-P + ADP ($\Delta G^{0'} = -23.8$ KJ/mol)

INTERCONVERSION OF ADENINE NUCLEOTIDES

The enzyme adenylate kinase (myokinase) is present in most cells. It catalyzes the interconversion of ATP & AMP on the one hand and ADP on the other.

(4) Adenosine – Ⓟ~Ⓟ~Ⓟ (ATP) + Adenosine – Ⓟ (AMP)

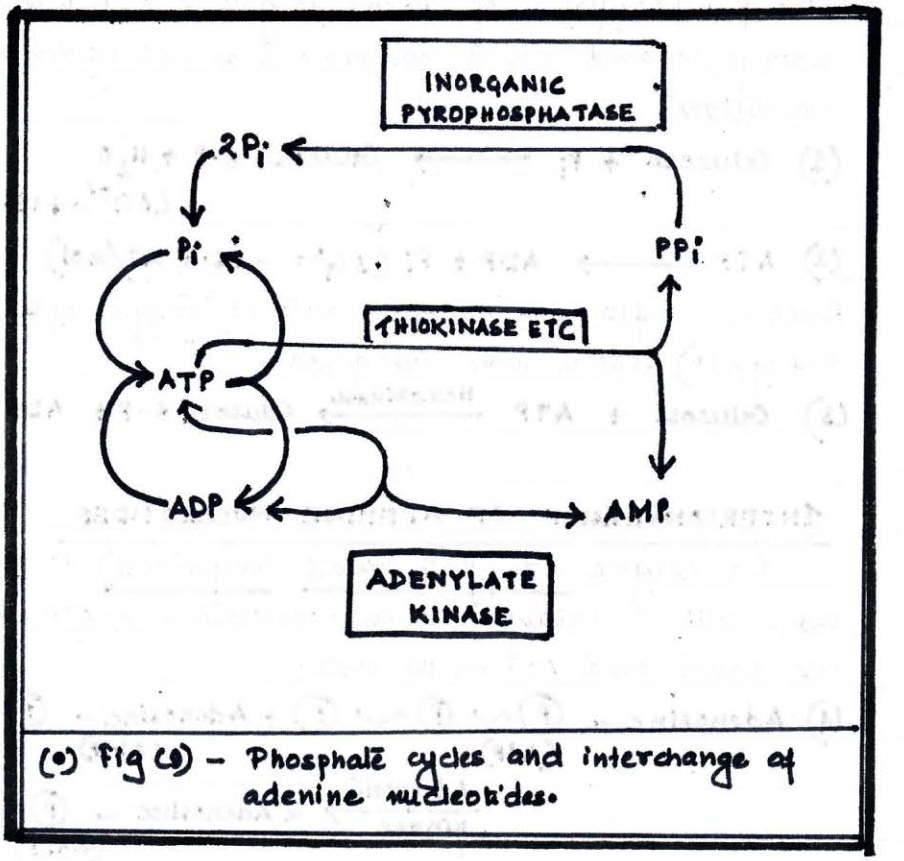
$\xrightarrow[\text{kinase}]{\text{Adenylate}}$ 2 Adenosine – Ⓟ~Ⓟ (2ADP)

When ATP reacts to form AMP, inorganic pyrophosphate (PP_i) is formed, as occurs – [activation of long chain fatty acids].

(5) ATP + CoA · SH + R · COOH $\xrightarrow{\text{Thiokinase}}$ AMP + PP_i + R · CO~S CoA

($\Delta G^{0'} \rightarrow -1.6$ Kcal/mol)

(6) $PP_i + H_2O \xrightarrow[\text{pyrophosphatase}]{\text{inorganic}}$ 2Pi



(•) Fig (9) – Phosphate cycles and interchange of adenine nucleotides.

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A combination of the above reactⁿs makes it possible for phosphate to be recycled and the adenine nucleotides to interchange (fig 9.)

Nucleoside Phosphates Related to ATP & ADP.

By means of the enzyme nucleoside diphosphate kinase, nucleosides triphosphates similar to ATP but containing a different base from adenine, can be synthesized from their diphosphates. e.g

{Nucleoside Diphosphate kinase}

ATP + UDP ⟷ ADP + UTP (uridine triphosphates)

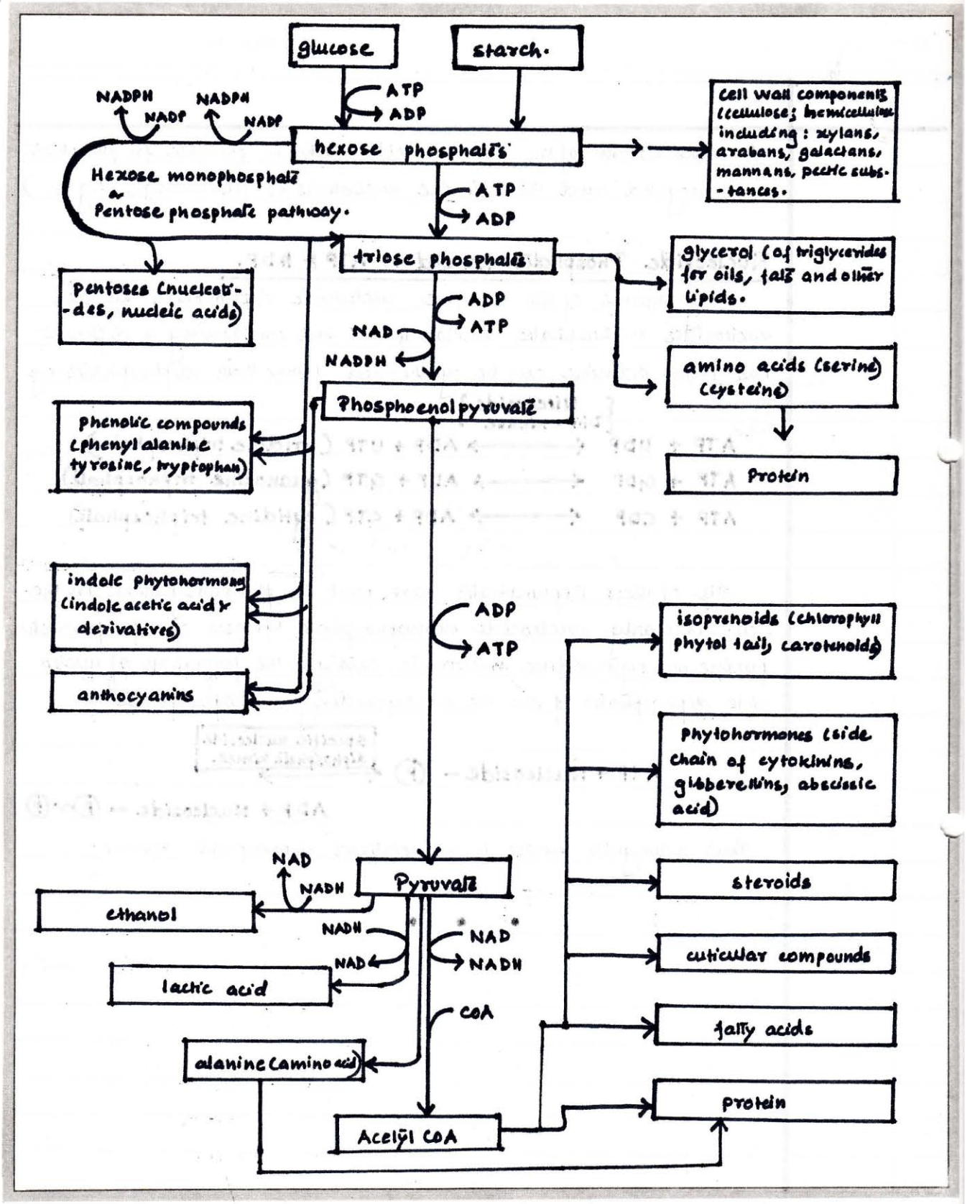
ATP + GDP ⟷ ADP + GTP (guanosine triphosphate)

ATP + CDP ⟷ ADP + CTP (cytidine triphosphate)

All of these triphosphates take part in phosphorylations in the cell. Similarly nucleoside monophosphate kinases, specific for each purine or pyrimidine nucleoside, catalyze the formation of nucleoside diphosphates from the corresponding monophosphates.

ATP + Nucleoside – Ⓟ ⟷ (specific nucleoside diphosphate kinase) ADP + Nucleoside – Ⓟ~Ⓟ

Thus adenylate kinase is a specialized diphosphate kinase.


glucose
starch.
ATP
ADP
NADPH
NADP
NADPH
NADP
hexose phosphates
cell wall components (cellulose; hemicelluloses including: xylans, arabans, galactans, mannans, pectic substances.
Hexose monophosphate & Pentose phosphate pathway.
ATP
ADP
pentoses (nucleotides, nucleic acids)
triose phosphates
glycerol (of triglycerides for oils, fats and other lipids.
ADP
ATP
NAD
NADH
amino acids (serine) (cysteine)
Phosphoenolpyruvate
Protein
phenolic compounds (phenylalanine tyrosine, tryptophan)
indole phytohormones (indole acetic acid & derivatives)
anthocyanins
ADP
ATP
isoprenoids (chlorophyll phytol tail, carotenoids)
phytohormones (side chain of cytokinins, gibberellins, abscissic acid)
NAD
NADH
Pyruvate
steroids
ethanol
NADH
NAD
NAD
NADH
cuticular compounds
lactic acid
CoA
fatty acids
alanine (amino acid)
protein
Acetyl CoA

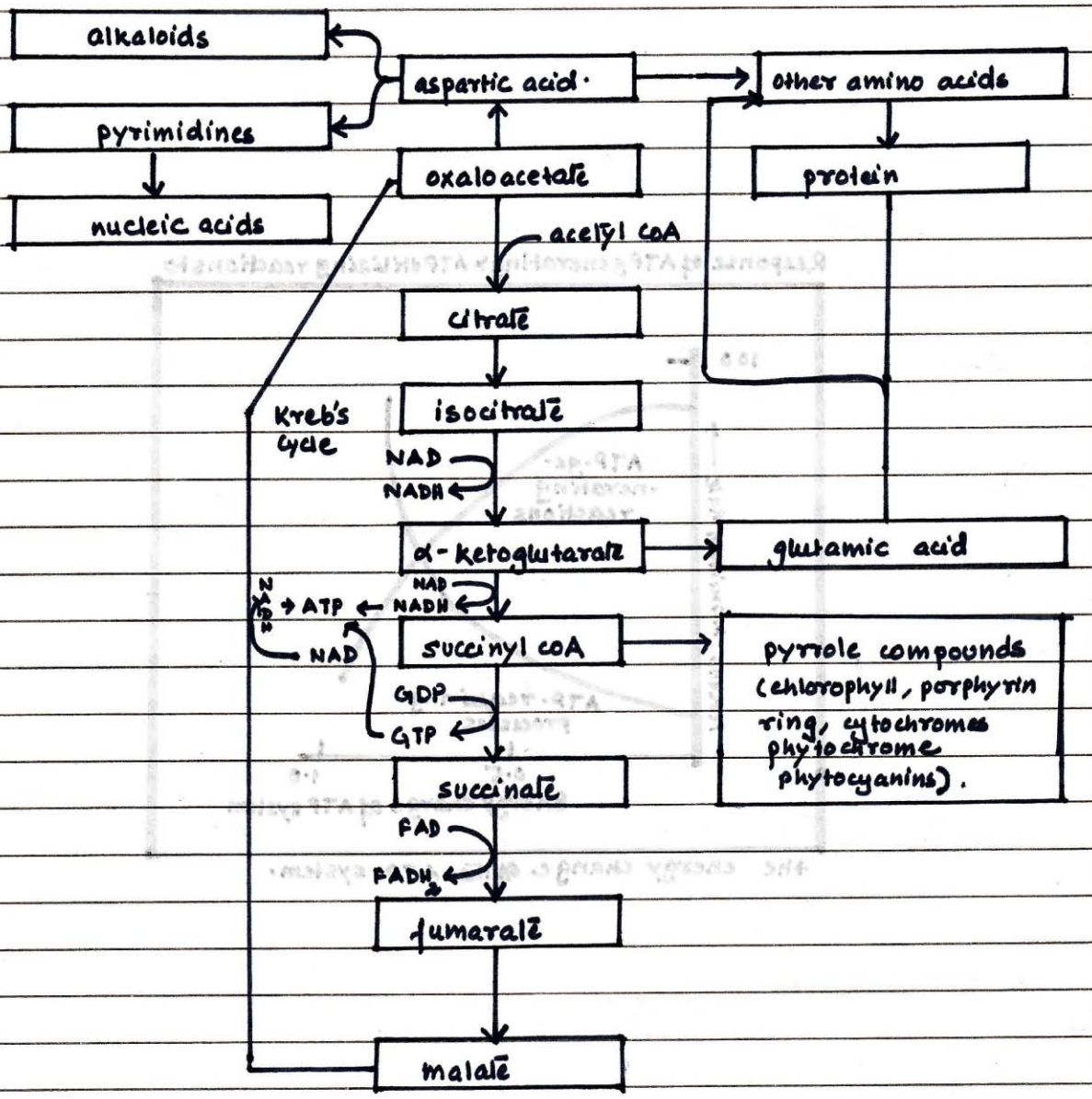
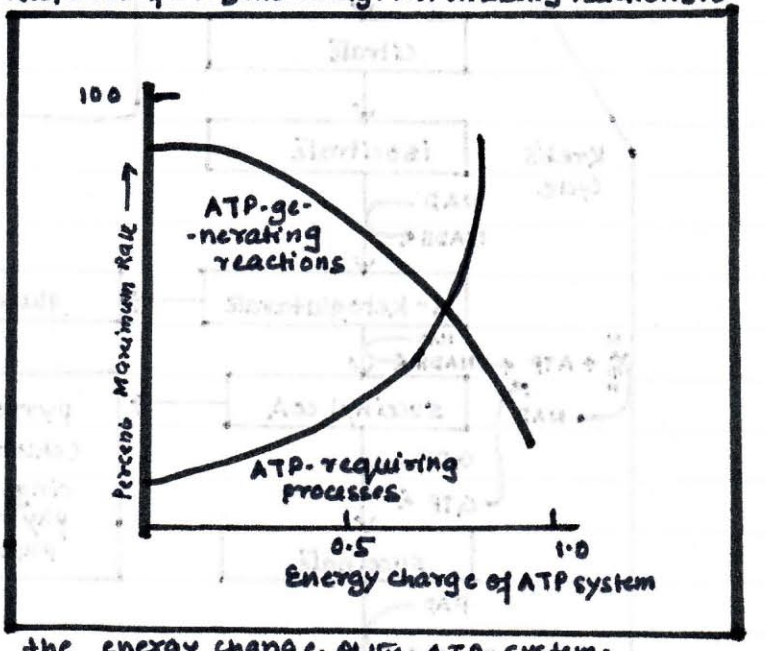


Fig aside x above :- Overview of Relationship of Cellular Components and Energy Yielding Reactions of Respiration --

"INTER METABOLIC RELATIONSHIPS"

Response of ATP generating & ATP utilizing reactions to

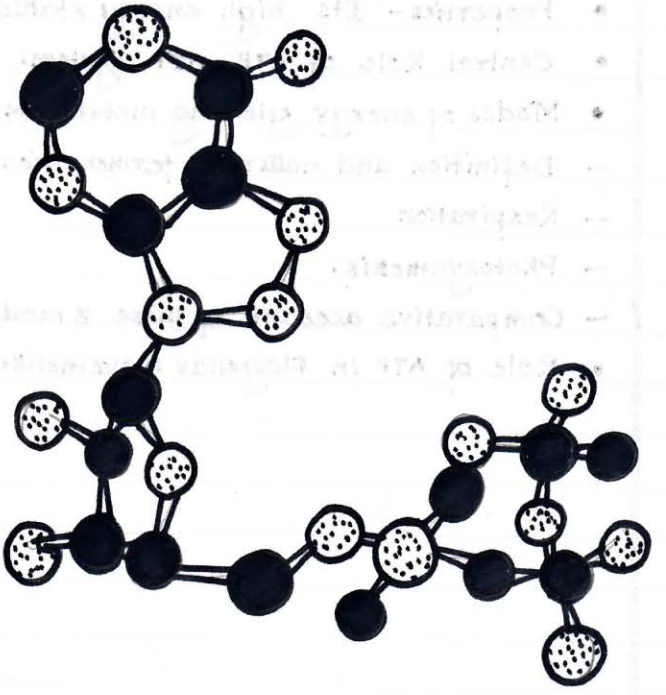


the energy change of the ATP system.

ATP - (Adenosine Triphosphate)

The Currency Of Cell....

Ball & Stick Model of Adenosine Triphosphate.



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ADENOSINE TRI-PHOSPHATE

"ATP"

(•) AN OVERVIEW :-

- Introduction
- Structure
- Properties - Its high energy status.
- Central Role of ATP - ADP system.
- Modes of energy yielding metabolism.
  - Definition and nature of fermentation
  - Respiration
  - Photosynthesis.
  - Comparative account of these 3 modes.
- Role of ATP in Flagellar movement.

ATP - The currency of cell

ATP - The energy carrier.

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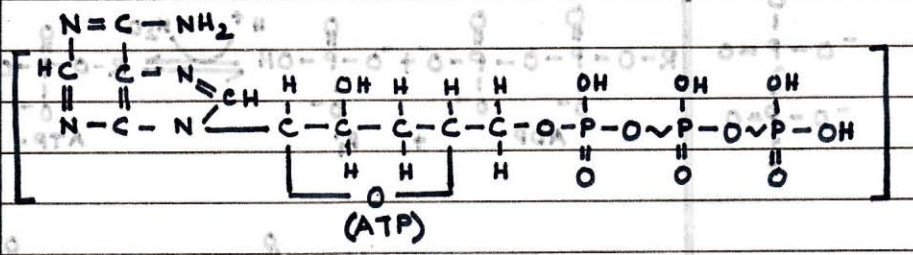
INTRODUCTION :

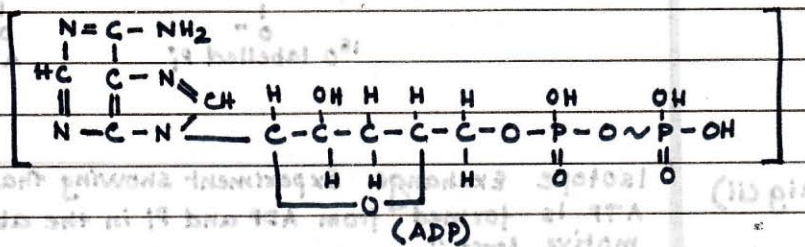
- ATP was first isolated from acid extracts of muscle in 1929 by Fiske and Subbarow.
- Its structure was deduced some years later by degradation experiments and ultimately confirmed by total chemical synthesis by Todd and his colleagues in 1948.
- From its first discovery ATP was suspected to play a role in cellular energy transfer, but it was not until 1939-1941 that Lipmann proposed it serves as a principal means of transfer of chemical energy in the cell.

STRUCTURE :

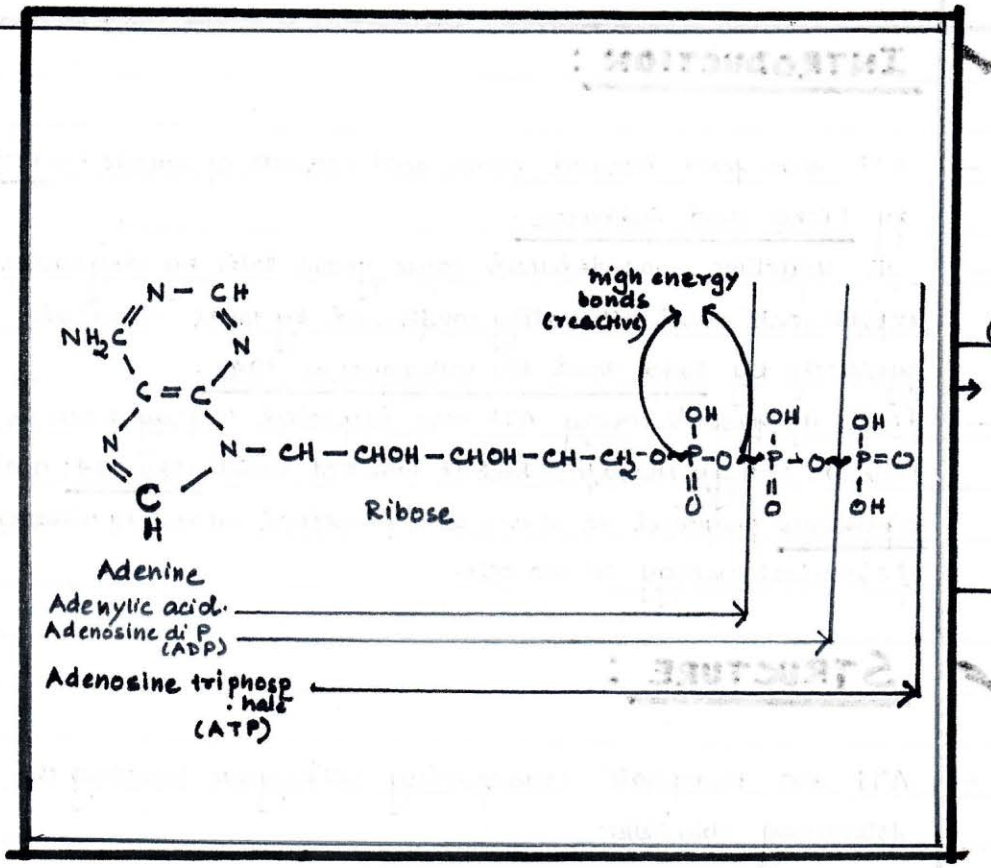
- ATP are phosphate-transferring coenzymes having the following structure.



(ATP)

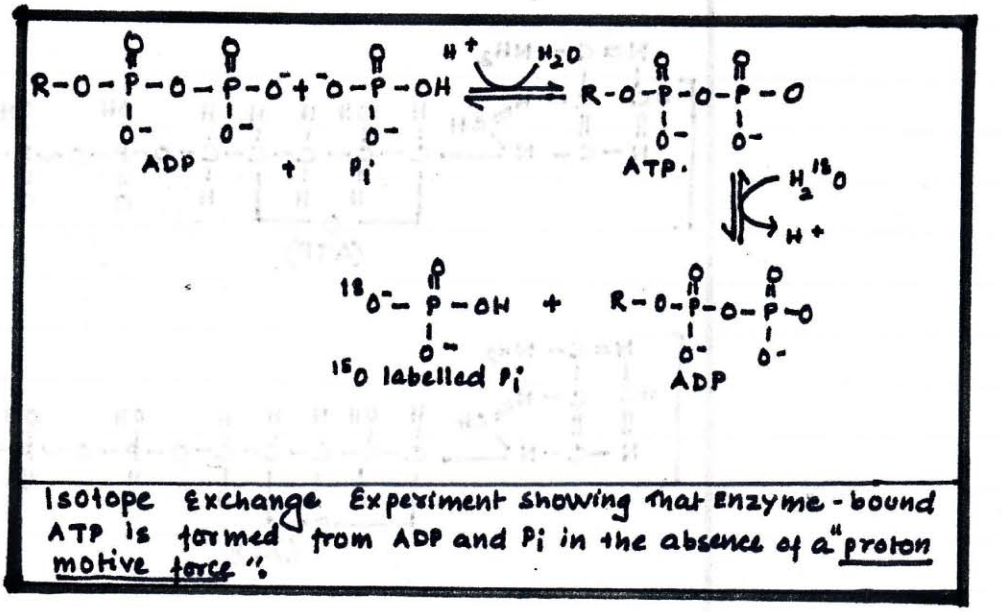


(ADP)



(*) Fig (i)

→ STRUCTURE OF ADENYLIC ACID AND PHOSPHATE DERIVATIVES ADP AND ATP.



(*) Fig (ii)

Isotope Exchange Experiment showing that Enzyme-bound ATP is formed from ADP and Pi in the absence of a "proton motive force".

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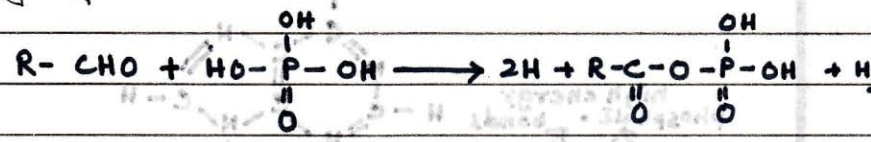
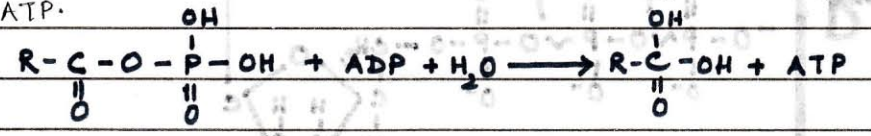
- ATP is the "universal fuel" of the living cell.
- It contains two high energy phosphate bonds (~) and each stores about 12,000 calories and releases about 7,500 calories when broken.
- ATP is produced by two series of reactions :-

(1) an aldehyde reacts with an inorganic phosphate to give hydrogen and an acid phosphate



(2) the acid phosphate reacts with ADP to give an organic acid & ATP.

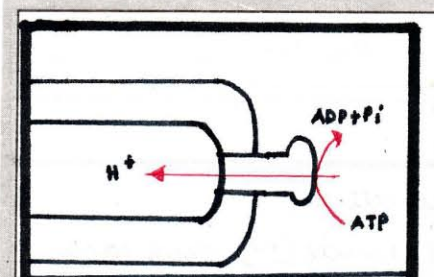


- ATP due to its high energy bonds and PO_4 groups is able to donate number of PO_4 groups to a number of metabolic linkages, thereby converting them to activated forms.
- Their increased free energy allows a phosphorylated intermediate to participate in biosynthetic reactions.
- The special reactivity of the high energy bonds of ATP is apparent when $\Delta G^{0\prime}$ (Free energy) of their hydrolysis is compared with the $\Delta G^{0\prime}$ of hydrolysis of the phosphate of AMP attached to adenosine by an ester linkage. Therefore less reactive and termed as low energy bonds.

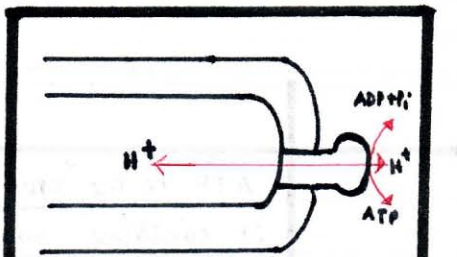
Adenosine – Ⓟ~Ⓟ~Ⓟ + H_2O → Adenosine – Ⓟ~Ⓟ + Ⓟ

ADP. $\Delta G^{0\prime} = -7.3$ Kcal.

The chemiosmotic theory of proton electrochemical coupling.

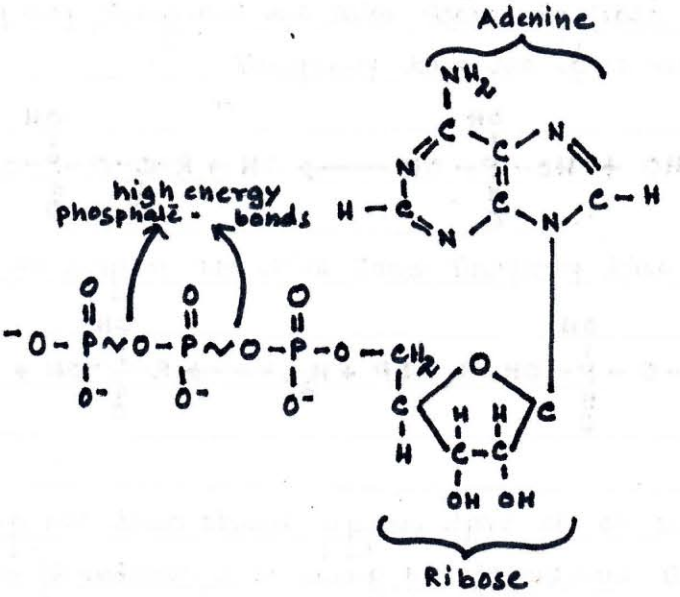


One proton pump: ATP hydrolysis drives protons across the pump. As H^+ builds up on the other side it sets up a potential gradient to drive ATP synthesis.



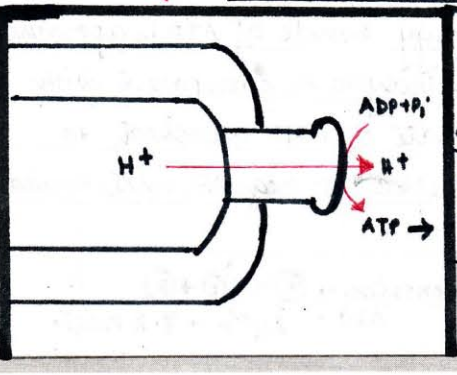
Equilibrium: between energy derived from ATP hydrolysis + energy needed to pump protons against an increasing gradient.

Coupling



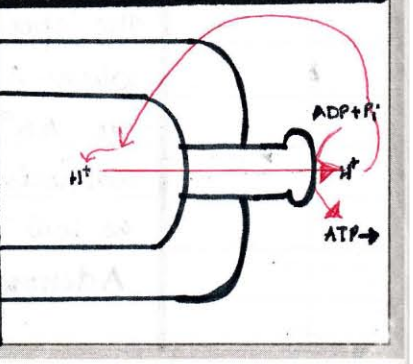
Coupling

• Fig (iii) — "ATP AS ENERGY CARRIER"



ATP removed as it is made the proton gradient drives ATP synthesis. This depletes the gradient, unless it is constantly replenished.

Second proton pump: This replenishes the proton gradient so that ATP synthesis can continue.



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Adenosine — Ⓟ ~ Ⓟ + H_2O → Adenosine — Ⓟ + Ⓟ

$\Delta G^{0\prime}_{AMP} = -7.3$ kcal.

PROPERTIES :

(1) Its high energy status : ATP as energy carrier.

A. Chemical reactions are coupled through common intermediates

- Two chemical reactions have a common intermediate when they occur sequentially so that the product of the first reaction is the substrate for the second.

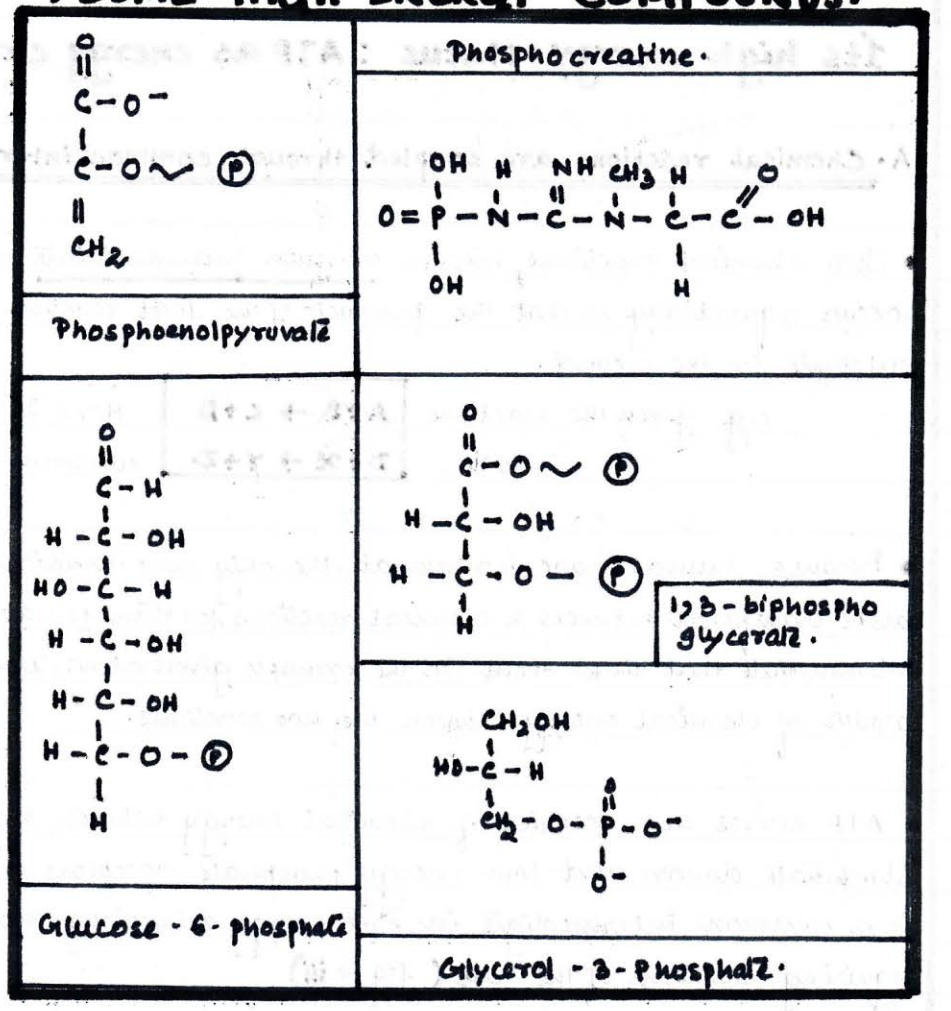
e.g. given the reactions $A + B \rightarrow C + D$ and $D + X \rightarrow Y + Z$ — Here 'D' is the common intermediate

- Because humans are isothermal, the only way in which energy can be transferred between 2 chemical reactions for them to have a common intermediate that links them. In the example given above, D could be a carrier of chemical energy between the two reactions.

- ATP serves as a carrier of chemical energy between high energy phosphate donors and low-energy phosphate acceptors because it is a common intermediate in both energy delivering and energy requiring reactions of the cell (fig → iii)

(Fig iv)

(e) :- SOME HIGH ENERGY COMPOUNDS :-



N·B → ENERGY CHANGE.

The relative amount of high energy forms of ATP (ATP, ADP) can be calculated using the following formula:

$$EC = \frac{1}{2}[ADP] + 2[ATP] / ([AMP] + [ADP] + [ATP]).$$

NOTE that if all adenosine phosphates are ATP, EC = 1·0 ; if all AMP, E·C = 0 ; & if all ADP or ATP = AMP, EC = 0·5

- **EC vs % maximum reaction rate**
- **The two roles for ATP**

FREE ENERGY AND ATP.

How does the energy in ATP specifically get utilized to power reactions in metabolism?

- The laws of Thermodynamics – First law : In any process, the total energy of the systems and the surroundings remains constant energy is not created nor destroyed, however can be transformed from one form to another.

Second Law. : In any process, the entropy of the system and the surroundings increases, Entropy is often thought of as disorder. or randomness.

THE ULTIMATE DRIVING MACHINE:

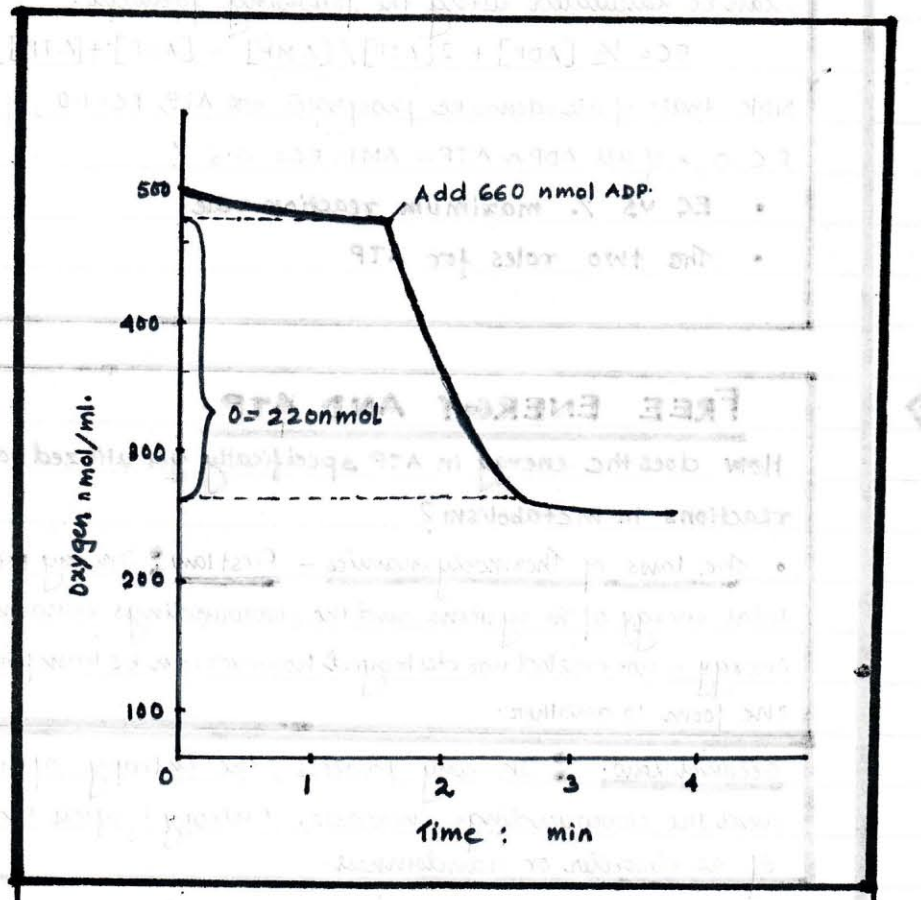
- A New Value for Predicting the Direction of chemical Reactions free energy

$\boxed{DG = DH - TDS}$

G = free energy
H = enthalpy (total energy contained in chemical comp.-ounds)
S = entropy
T = absolute temperature

$A + B \Leftrightarrow C + D$

$DG = DG^{\circ} + RT\ln([C][D]/[A][B])$.



(•) Determination of the P/O Ratio →

Liver mitochondria are incubated in the presence of glutamate. The rate of O_2 uptake from the medium measured by oxygen electrode, is initially low. If ADP is added, respiration speeds up until the ADP is phosphorylated to ATP. The latter can be measured as esterification of Pi. If all of the ADP is esterified, the P/O ratio is 600/220 = 2.7.

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B. The energy carried by ATP is stored in its two terminal phosphate groups.

- ATP is composed of a molecule of adenosine to which three phosphate groups are attached. If one phosphate is removed, ADP (Adenosine diphosphate is produced; if two phosphates are removed, AMP (adenosine monophosphate results).

- At physiologic P^H, ATP is highly negatively charged having a total of three or four negative charges on its phosphates. ATP therefore forms a stable complexes with Mg^{++} and Mn^{++}

- The standard free energy of hydrolysis ΔG°, is approximately -7300 cal/mole for each of the two terminal phosphate groups. Because of this large negative ΔG°, ATP is called a **high energy phosphate compound.**

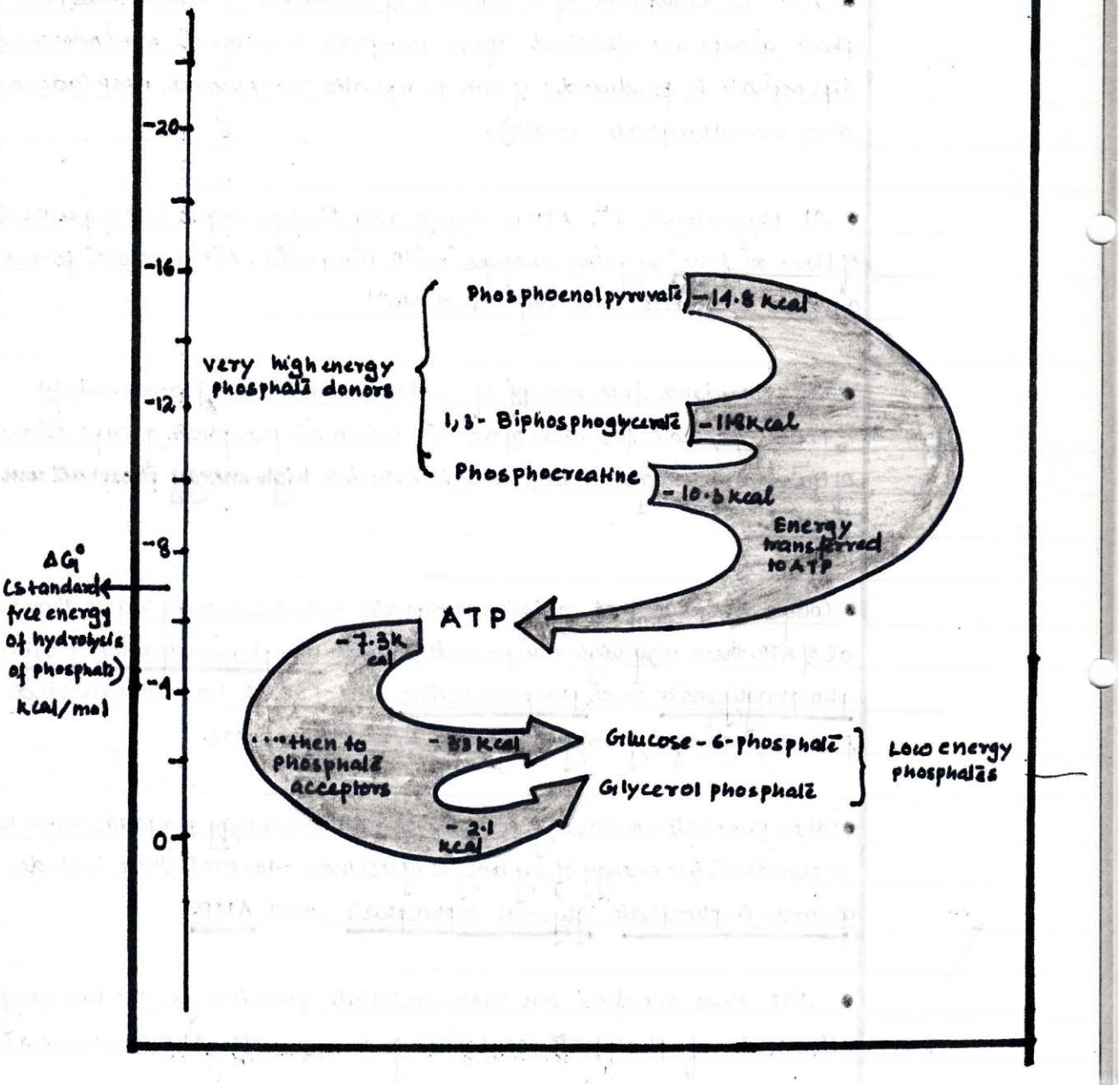
- Compounds exist that contain phosphates with an energy higher than that of ATP. These very high energy compounds include phosphoenolpyruvate, 1-3, bisphosphoglycerate and phosphocreatine, all of which have a standard free energy of hydrolysis greater than -10,000 cal.

- Other phosphate containing compounds have low energy phosphates which have standard free energy of hydrolysis of less than -4000 cal. These include glucose-6-phosphate, glycerol-3-phosphate and AMP.

- ATP thus occupies an intermmediate position on the bioenergetic scale of phosphate containing compounds. ADP can serve as

(Figv) (•)

ATP carries energy between high & low energy compounds.



an acceptor of phosphate groups from cellular phosphates containing higher energy phosphates. ATP can donate these phosphates to compounds in the cell forming phosphates of lower energy (fig V). There are no enzymes in cells that can transfer phosphate groups directly from very high-energy donors to low-energy acceptors without their first being transferred to ATP.

(•) ATP IS THE UNIVERSAL CURRENCY OF FREE ENERGY IN BIOLOGICAL SYSTEMS.

- The central role of ATP in energy exchanges in biological systems was perceived by **Fritz Lipman and by Herman Kalckar in 1941.**
- ATP is a nucleotide consisting of an adenine, a ribose and a triphosphate unit. In considering the role of ATP as an energy carrier, we can focus on its triphosphate moiety. ATP is an rich molecule because its triphosphate unit contains two phosphoanhydride bonds.

$$ATP + H_2O \rightleftharpoons ADP + P_i + H^+ \quad \{\Delta G^{0'} = -7.3 \text{ kcal/mol}\}$$

$$ATP + H_2O \rightleftharpoons AMP + PP_i + H^+ \quad \{\Delta G^{0'} = -7.3 \text{ kcal/mol}\}$$

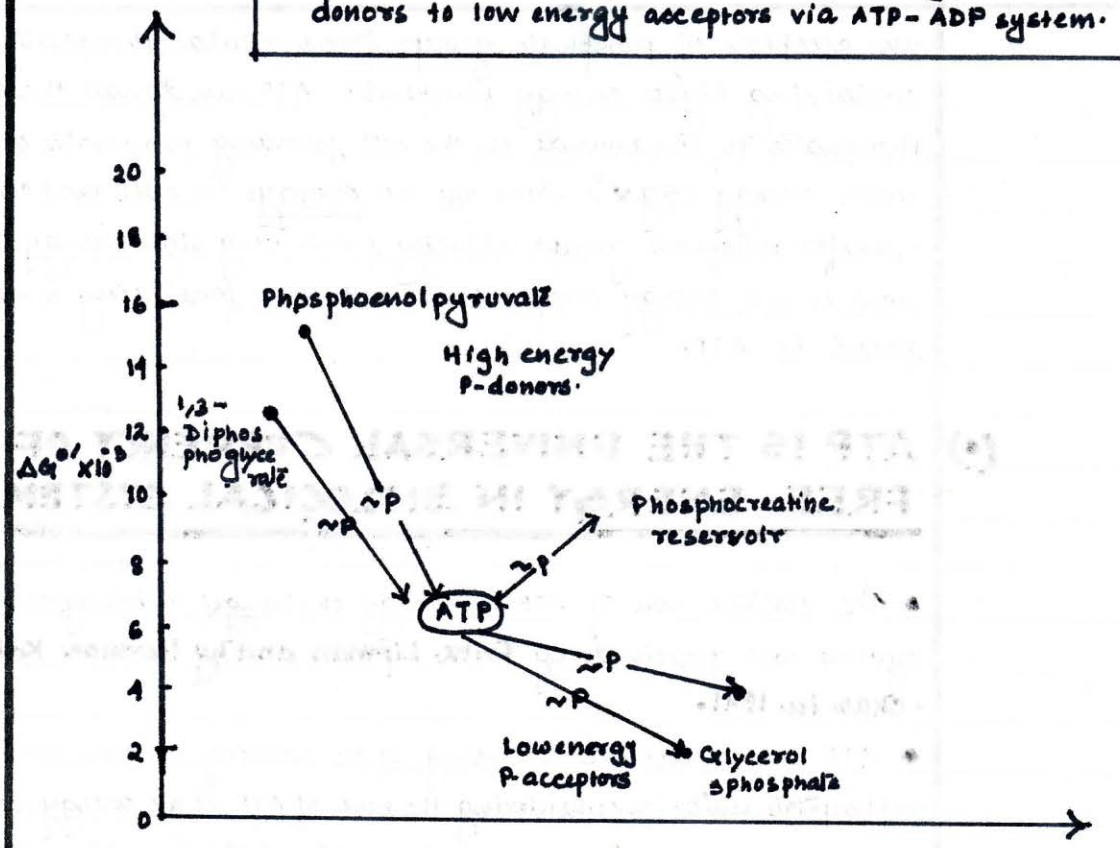
- ATP, AMP and ADP are interconvertible. The enzyme adenylate kinase (myokinase) catalyzes the reaction.

$$ATP + AMP \rightleftharpoons ADP + ADP$$

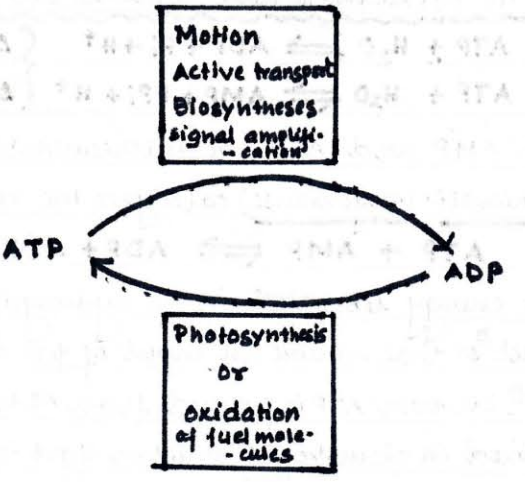
The free energy liberated in the hydrolysis of ATP is harnessed to drive reactions that require an input of free energy, such as muscle contraction. In turn ATP is formed from ADP & P_i when fuel molecules are oxidized in chemotrophs or when light is trapped by phototrophs. **This ATP-ADP cycle is the fundamental mode of energy exchange in biological systems.**

(Fig vi) →

(•) Flow of phosphate groups from high-energy phosphate donors to low energy acceptors via ATP-ADP system.



(Fig vii) →



(•) The ATP-ADP cycle is the fundamental mode of energy exchange in biological systems

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CENTRAL ROLE OF ATP-ADP CYCLE

:ATP IS CONTINUOSLY FORMED AND CONSUMED :-

- ATP serves as the principle immediate donor of free energy in biological systems rather than as a long term storage form of free energy.

- In a typical cell, an ATP molecule is consumed within a minute following its formation. **the turnover of ATP is very high.**

- Motion, active transport, signal amplification and biosyntheses can occur only if ATP is continuously regenerated from ADP. (fig vii)

- Phototrophs harvest the free energy in light to generate ATP, whereas chemotrophs from ATP by the oxidation of fuel molecules.

- In effect an ATP/ADP cycle connects those processes which generate ~ Ⓟ to those processes that utilize ~ Ⓟ

- The processes that feed ~ Ⓟ into this cycle involves -.

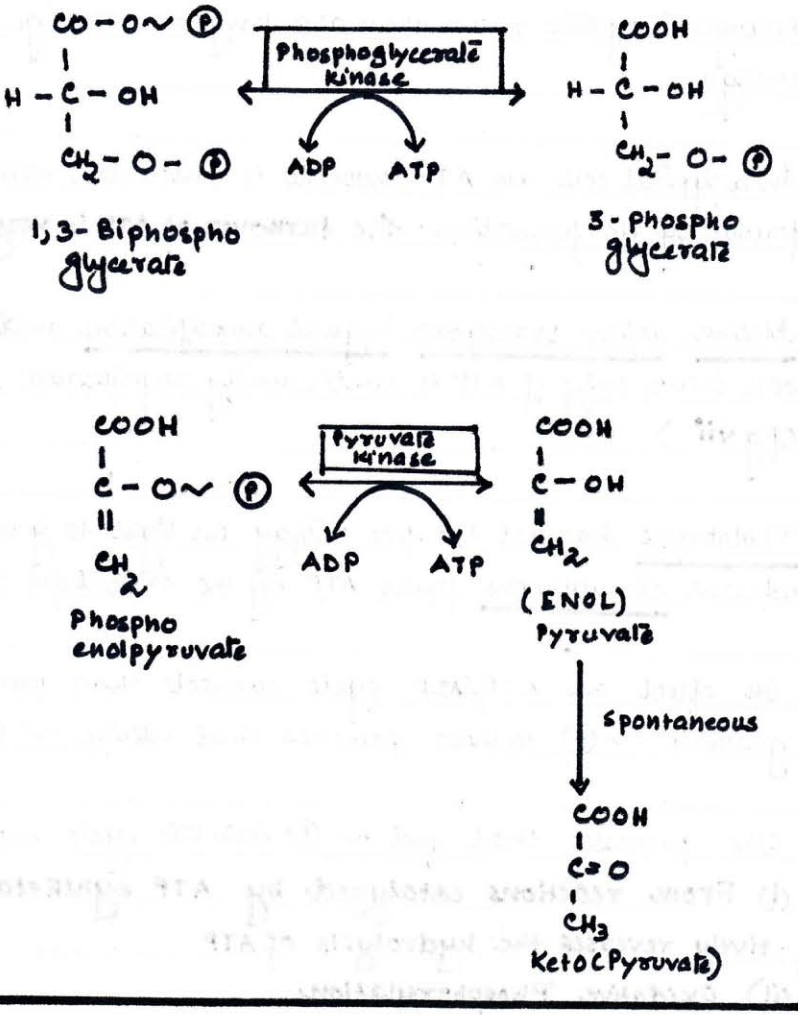
(i) From reactions catalyzed by ATP synthetase which effectively reverses the hydrolysis of ATP

(ii) Oxidative Phosphorylation

(iii) Embden Myerhof Parnas Pathway

(iv) Incorporation of P_i into 3-phosphoglyceraldehyde which after dehydrogenation forms 1,3-biphosphoglycerate.

(Fig viii) (a) Transfer of high energy phosphate from intermediates of EMP to ADP.



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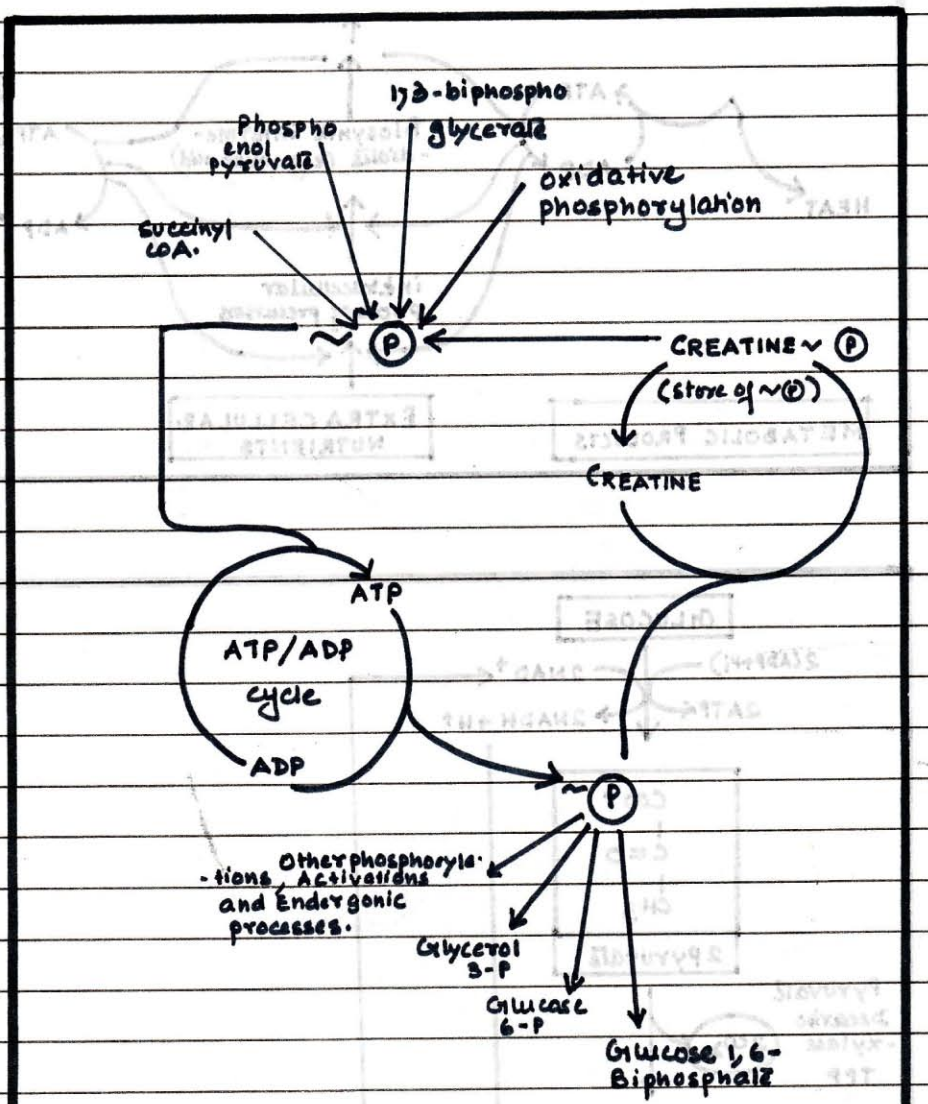
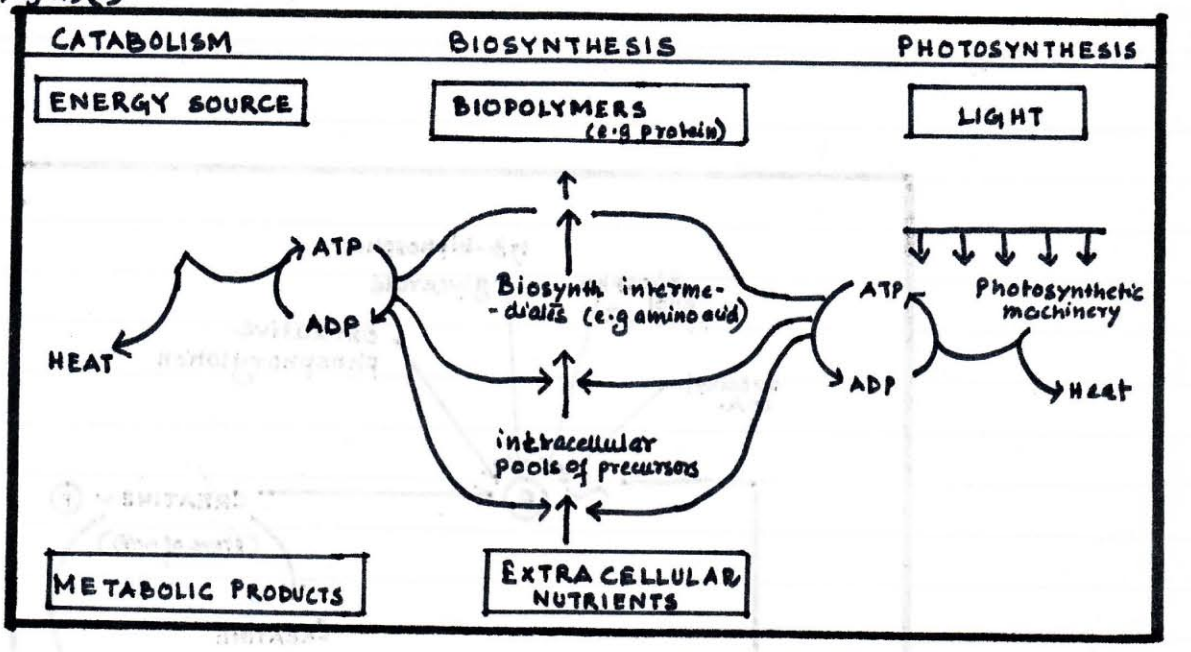
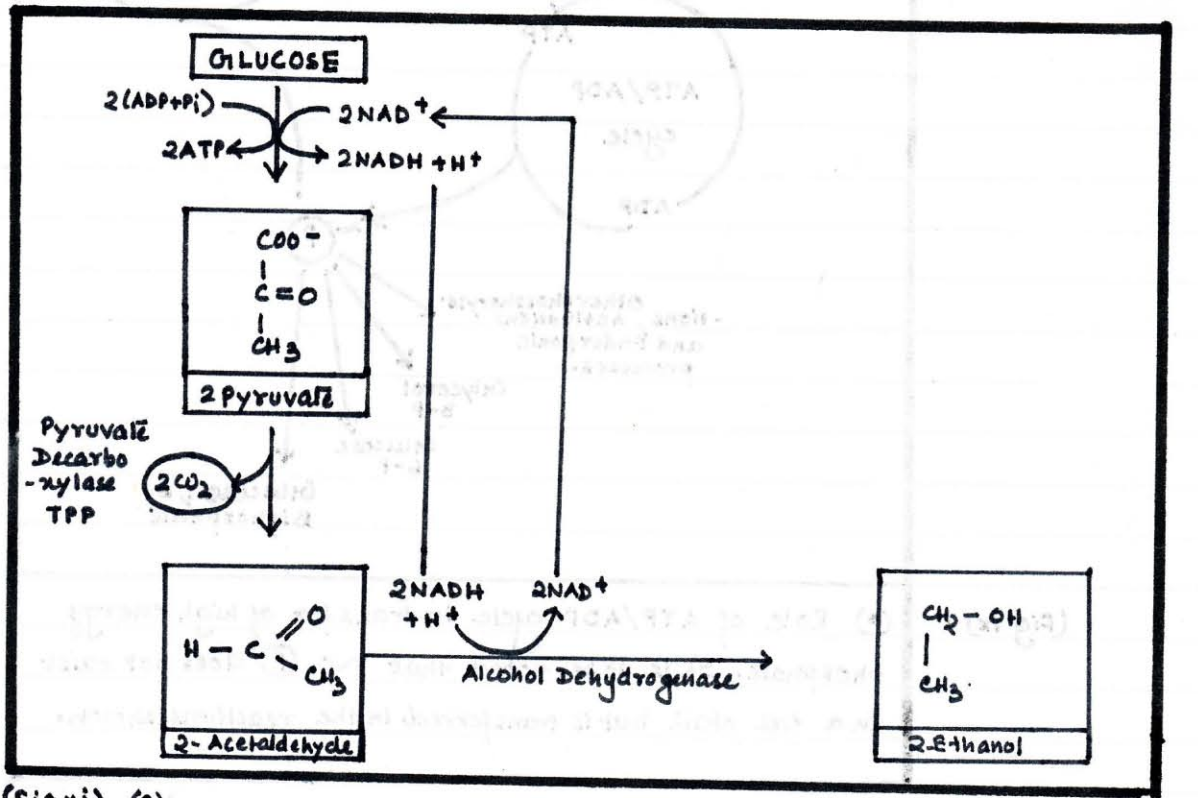


(Fig ix). (•) Role of ATP/ADP cycle in transfer of high energy phosphate. It is to be noted that ~ (P) does not exist in a free state but is transferred in the reactions shown.

(Fig x) (•)





(Fig xi) (•) YIELD OF ATP FROM ALCOHOLIC FERMENTATION.

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Modes Of Energy Yielding Metabolism.

— Generation of ATP is the fundamental mechanics by which some free energy can be trapped.

— In fact most is dissipated in the form of heat. The role of ATP in coupling energy to biosynthesis is summarised in the fig (✱.) aside.

COMPARISON OF FERMENTATⁿ/RESPIRATⁿ/P.Synthesis.

- **Fermentation → Its Nature and Definition.**

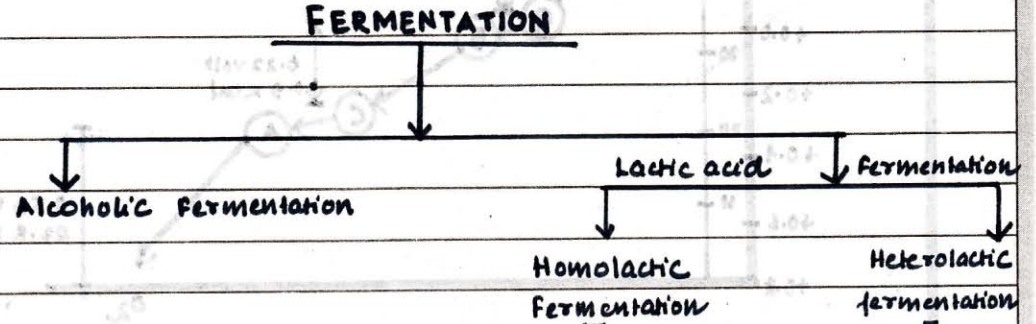
— In the strict sense, the term fermentation refers to those energy yielding pathways in which organic compounds act as both electron donors and electron acceptors

— During fermentation micro-organisms obtain energy from organic compounds without utilizing oxygen.

— The process of fermentation take place in two stages:

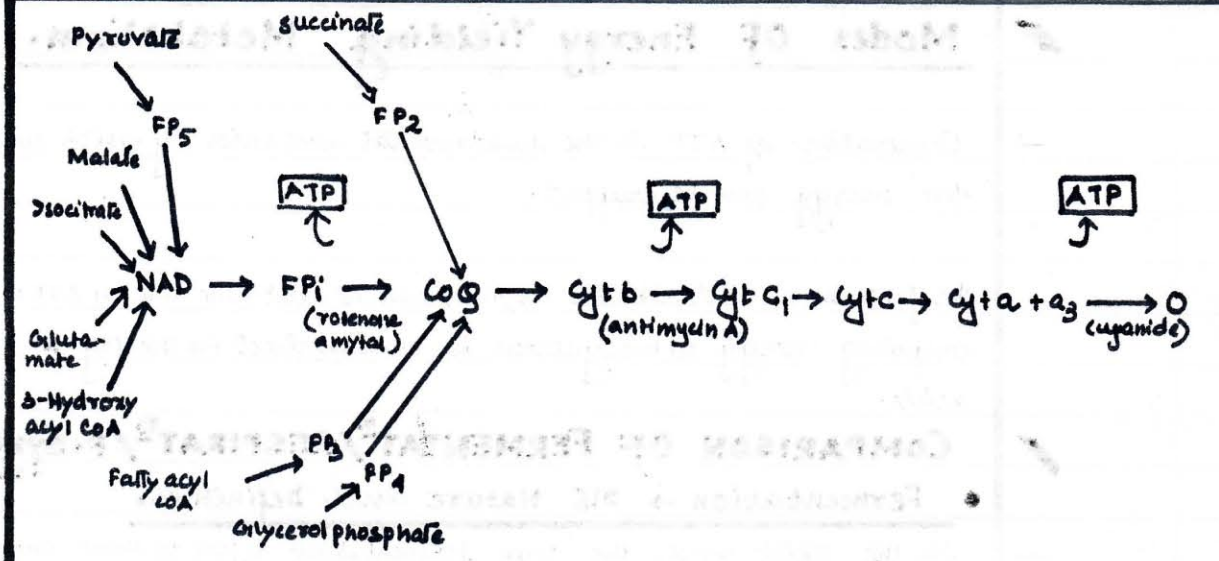
(1) Glucose is broken down to pyruvate with the release of two pairs of hydrogen atoms

(2) Pyruvate or compounds derived from pyruvate are reduced by the hydrogens released in the first stage.



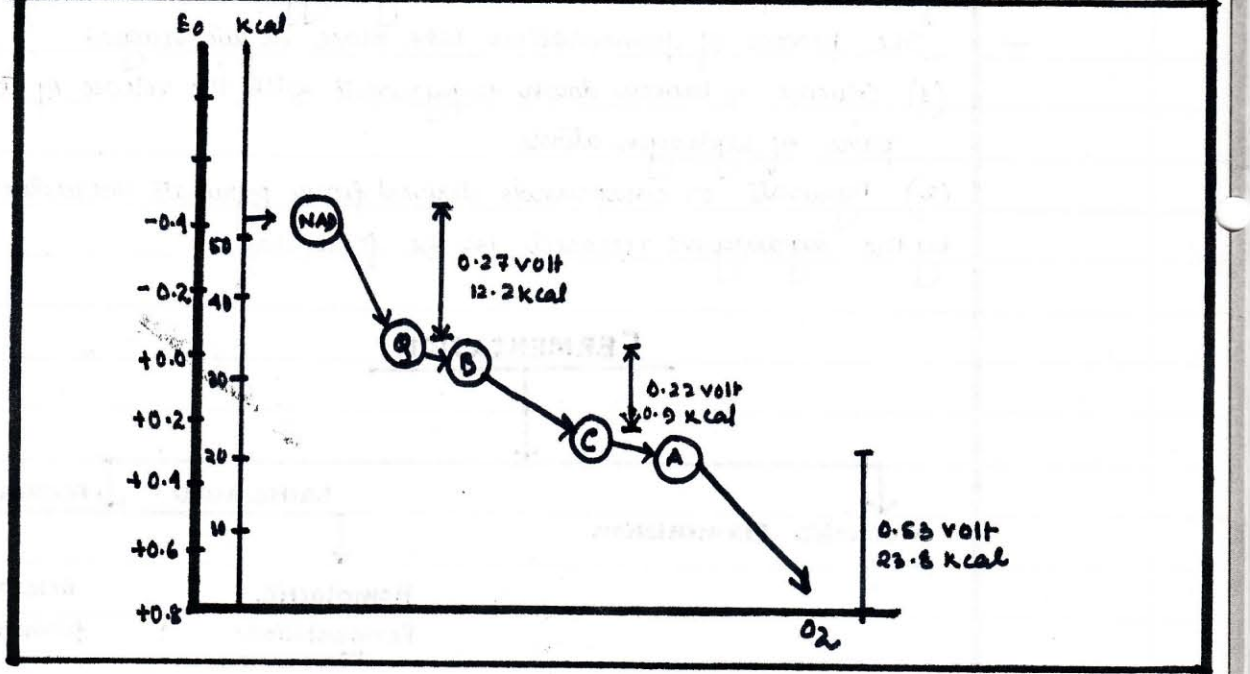
(Fig xii)

(•) The respiratory chain and the pts of entry of electrons from various substrates. Also shown the probable sites of energy conservation leading to ATP formation.



(Fig xiii)

(•) The decline in free energy as electron pairs flow down the respiratory chain to oxygen. Each of the three segments denoted in color yields sufficient energy to generate a molecule of ATP from ADP and phosphate



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RESPIRATION

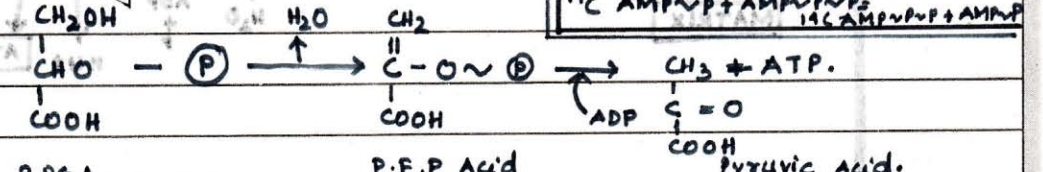
— Respiration is another major energy yeelding reactn

→ Oxidative Phosphorylation

→ Electron transport chain.

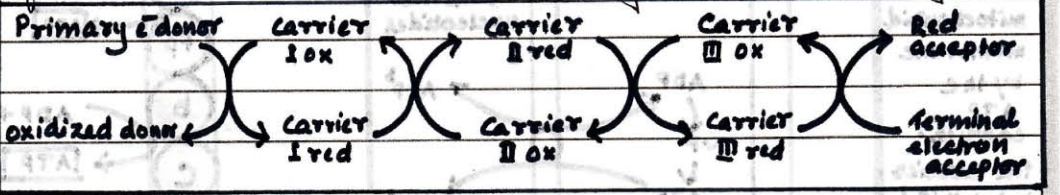
(•) **Synthesis from substrate level Phosphorylation**

— ATP is formed from ADP by transfer of ΔG° PO_4 gr in substrate level phosphorylation



(•) **Synthesis by ETC.**

— ATP is synthesized by transporting electrons through a carrier of molecules with fixed orientation in the cell membrane is a number of microbial metabolic process including respiration & Photosynthesis



— Each number of the chain is capable of being reduced by reacting with the carrier molecule that proceeds it and oxidized by the carrier that follows it.

Partial reactions of oxidative phosphorylation:

The isotopically labelled component is represented. AMP~P~P represents ATP > AMP~P → ADP.

1) ATPase activity.

$ATP + HOH \rightleftharpoons ADP + P_i$

2) ATP Phosphate exchange

$AMP\sim P\sim P + {}^{32}P \rightleftharpoons AMP\sim P_{\sim}{}^{32}P + P_i$

3) Phosphate water oxygen exchange

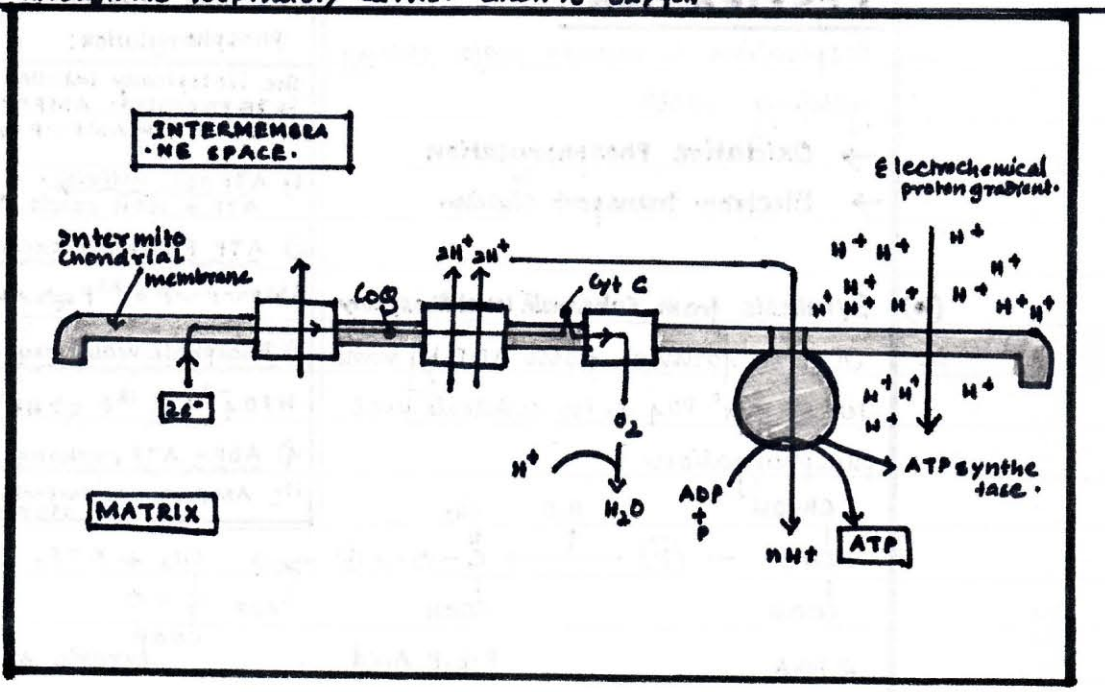
$HPO_4^{-2} + H_2{}^{18}O \rightleftharpoons HP^{18}O_4^{-2} + H_2O$

4) ADP - ATP exchange.

$^{14}C\ AMP\sim P + AMP\sim P\sim P \rightleftharpoons {}^{14}C\ AMP\sim P\sim P + AMP\sim P$

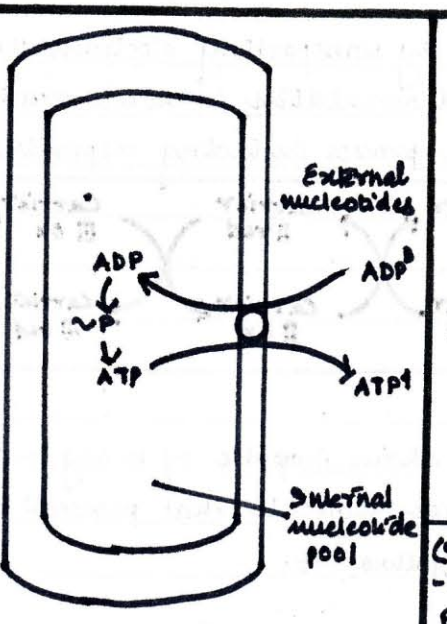
(a) Schematic illustration of the coupled processes of electron transport and oxidative phosphorylation. Using the proton motive force of the electrochemical proton gradient generated by the pumping of protons across the mitochondrial inner membrane. ATP synthetase catalyzes the synthesis of one ATP molecule for each pair of protons pumped out. In this way 3 molecules of ATP are made for the 3 pairs of electrons pumped out as one pair of electrons is transported through the respiratory carrier chain to oxygen

(Fig xiv)

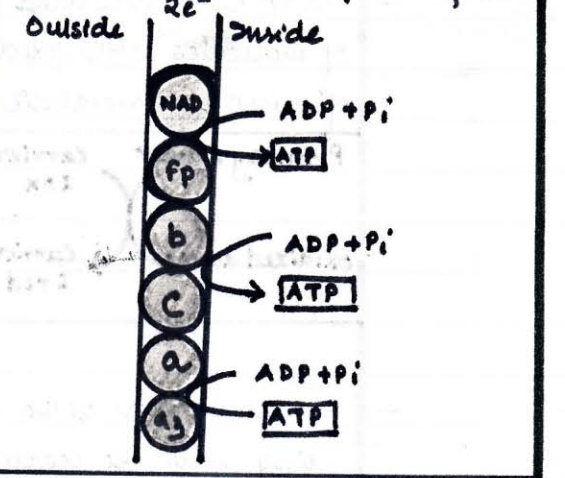


(Fig xv)

(a) Exchange of ADP & ATP across the inner mitochondrial membrane by the ATP carrier. The carrier is inhibited by very low conc^ns of atractyloside which bears some resemblance to the ATP molecule



Oxidative Phosphorylation of ADP



(a) Phosphorylation of ADP and accumulation of cations are alternative processes during electron transport.

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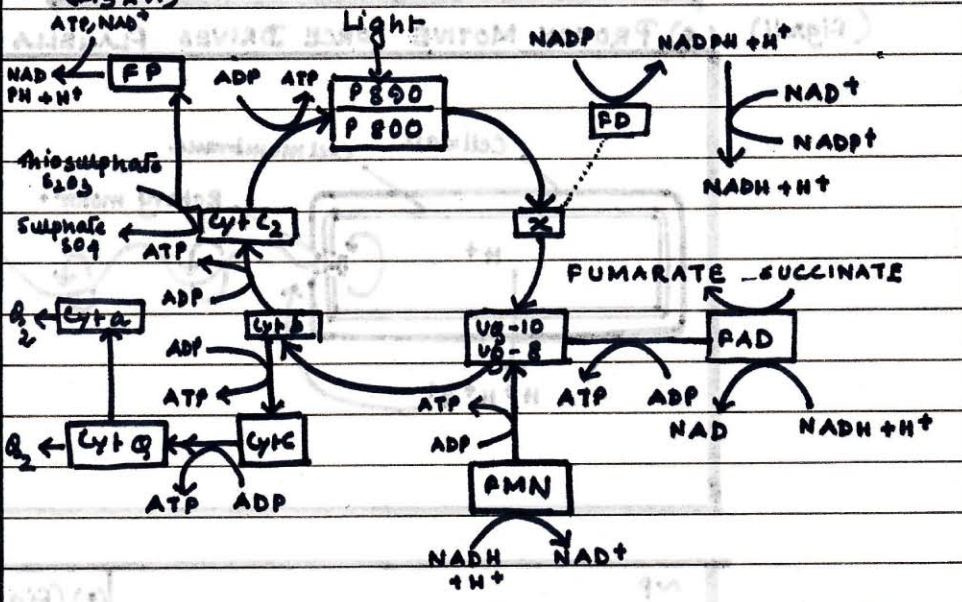
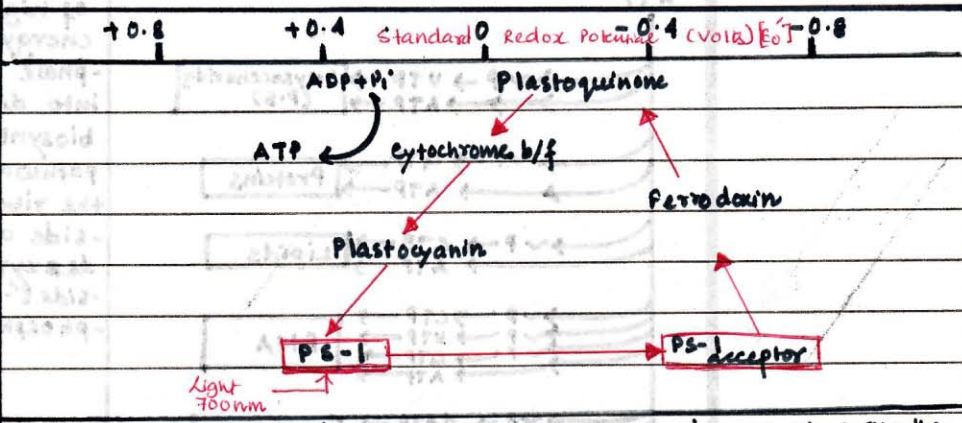
Photosynthesis →

Schematic Representation for electron transport in photosynthetic bacteria under aerobic & anaerobic conditions :-

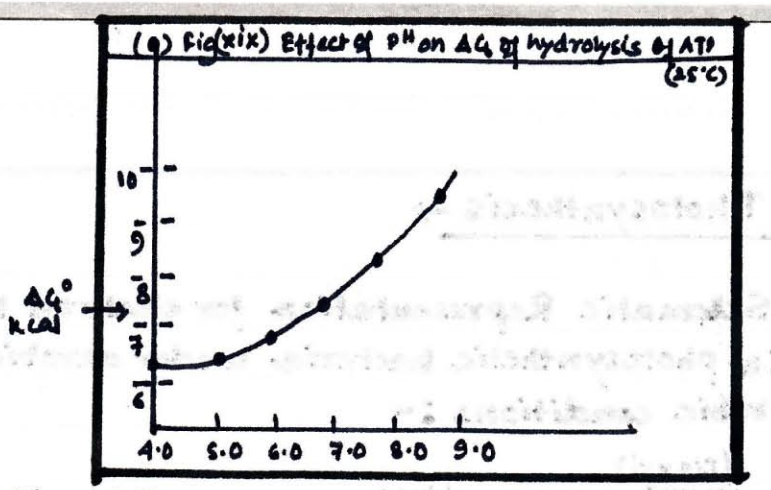
(Fig xvi)



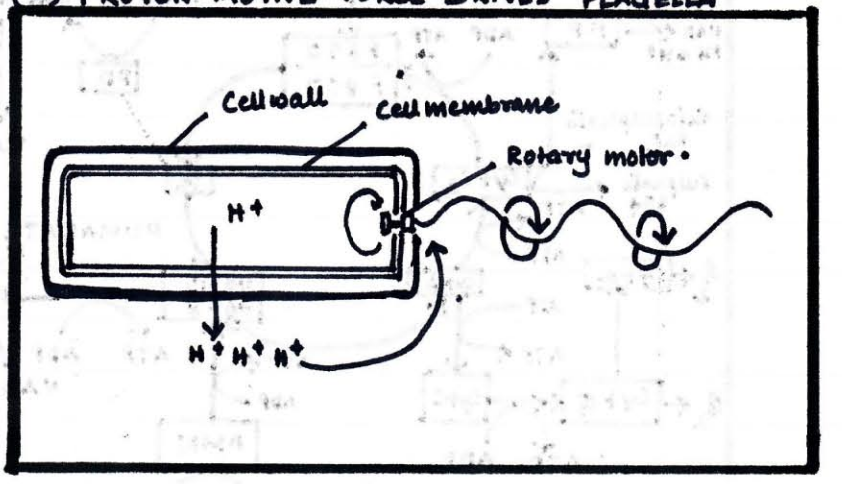


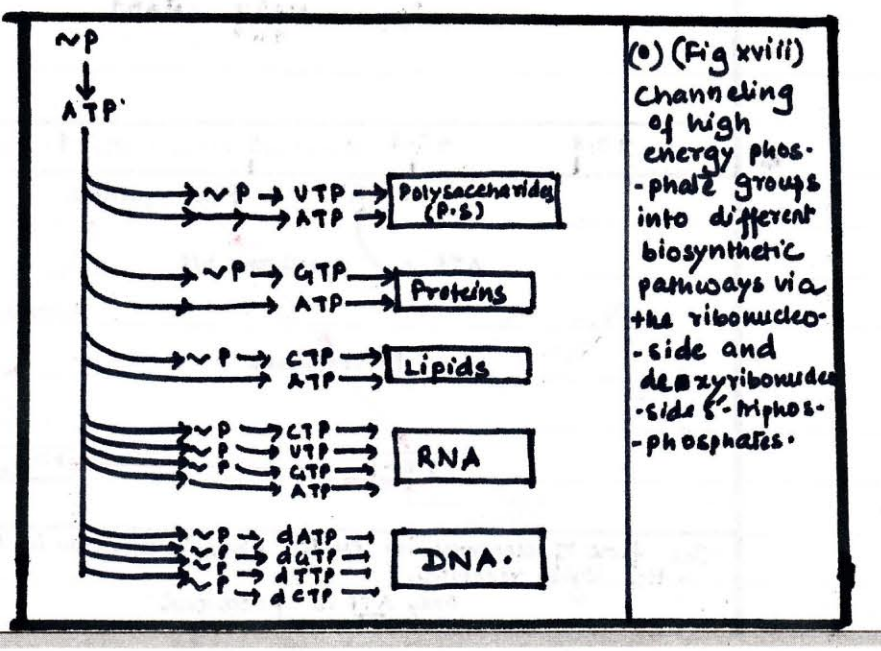
The flow of electrons in cyclic phosphorylation in the photosynthetic light reaction.
Only ATP is produced.

(e) Fig(xix) Effect of pH on ΔG of hydrolysis of ATP (25°C)



(Fig xvii) (e) PROTON MOTIVE FORCE DRIVES FLAGELLA





(e) (Fig xviii) Channeling of high energy phos-phate groups into different biosynthetic pathways via the ribonucleo-side and deoxyribonucle-side 5'-triphos-phosphates.

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ROLE OF ATP IN FLAGELLAR MOVEMENT:-

— Bacterial flagella filaments appear to have no machinery for interconverting chemical and mechanical energy. For example **flagellin**, the flagellar protein molecule, has no enzymatic activity i.e no detectable ATPase activity (such as is present in cilia and flagella of eucaryotic micro-organisms) (Fig xvii) → aside.

- **It is therefore a misconception that in prokaryotic flagellar movement ATP plays a role. Rather here "proton motive force" comes into play**
- **Generally in eukaryotic ciliary & flagellar movement ATP may play a significant role.**

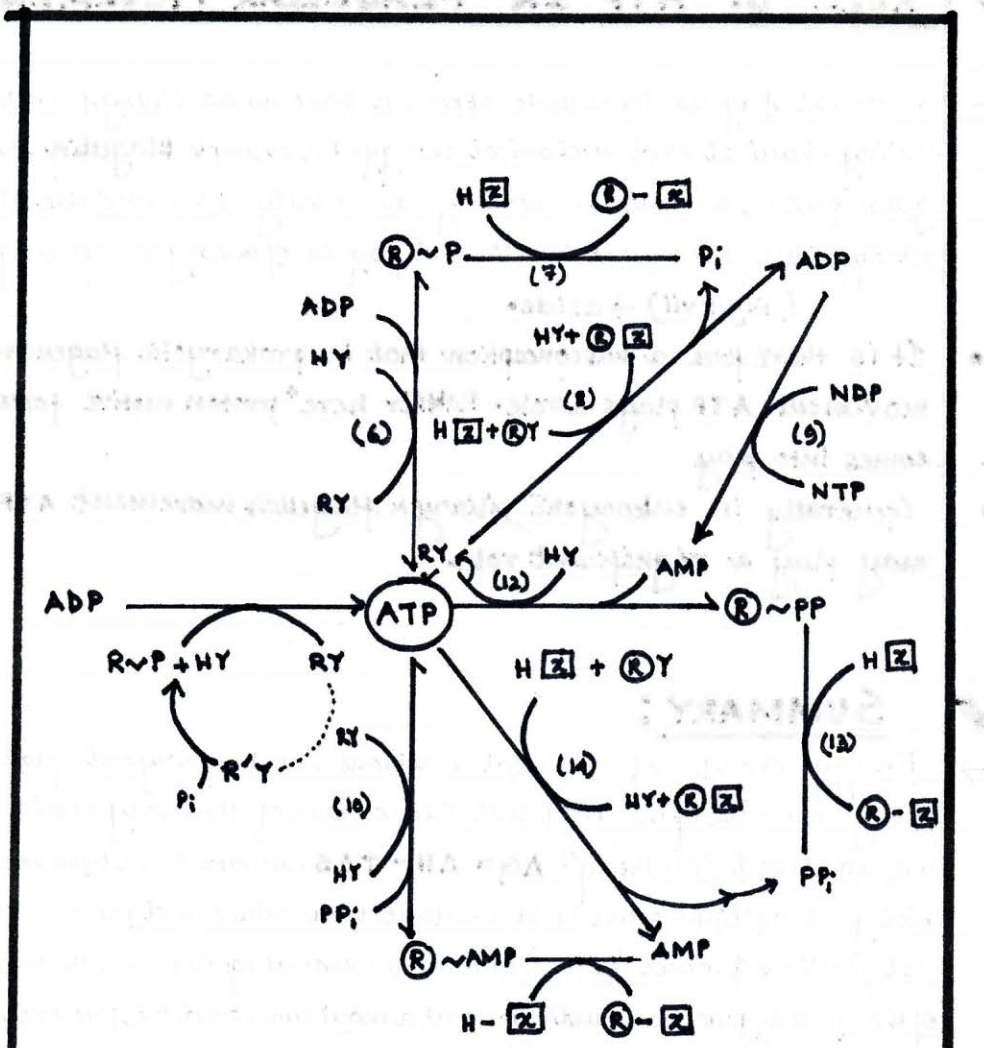
SUMMARY:

→ Energy changes of chemical reactions can be analyzed quantitatively in terms of the First and Second laws of thermodynamics, which are combined into the eqⁿ **$\Delta G = \Delta H - T\Delta S$**. Under conditions in which biological reactions occur i.e at constant temperature and pressure, chemical reactions proceed in such a direction that at equilibrium the entropy's of the system plus surroundings is at a maximum and the free energy G of the system alone is at a minimum. Every chemical reaction has a characteristic standard free energy G_T of the system alone is at a minimum. Standard temperature and pressure with all reactants and products at 1 M concⁿ and $P^H = 7$.

→ ATP is the energy currency of cell.

→ ATP is generated by Respiration, Photosynthesis and Fermentation

→ ATP is vital for all biological life processes.



(o) Fig(xx) Schematic Representation of the functions of ATP. Numbers in parentheses refer to reactions in Table given aside (Fig xxi) Bonds designated by ~ are characterized by large negative $\Delta G^{o'}$ of hydrolysis. N refers to a nucleoside.

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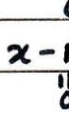
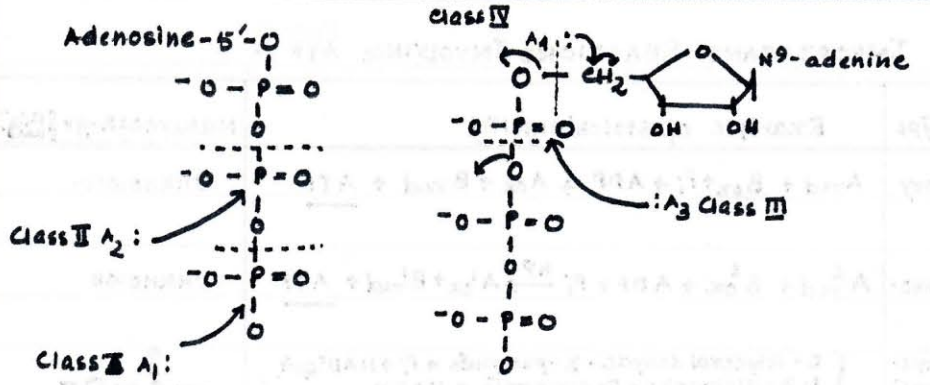
(*)(Fig xxi) :- IMPORTATANT REACTIONS INVOLVING ATP :-

Reaction or reaction type	Example of stoichiometry	Nature of R-gr [Fig]
Oxidative phosphorylation.	$A_{red} + B_{ox} + P_i + ADP \rightarrow A_{ox} + B_{red} + \underline{ATP}$	Unknown.
Photosynthetic phosphorylation.	$A^1_{red} + B^1_{ox} + ADP + P_i \xrightarrow{h\nu} A^1_{ox} + B^1_{red} + \underline{ATP}$	Unknown
Triose-Phosphate dehydrogenase plus glycerate kinase	D-Glyceraldehyde-3-phosphate + P_i + NAD^+ ⇌ 1,3-diphospho-D-glycerate + NADH 1,3-Diphospho-D-glycerate + ADP ⇌ 3-phospho-D-glycerate + ATP	
Enolase (phosphoenol pyruvate hydratase)	2-Phospho-D-glycerate ⇌ phosphoenolpyruvate + H_2O Phosphoenol pyruvate + ADP ⇌ pyruvate + ATP	
α-Oxoglutarate dehydrogenase plus succinate: CoA ligase (GDP) plus nucleoside diphosphate kinase or plus succinate: CoA ligase (ADP)	α-oxoglutarate + NAD^+ + CoASH → succinyl-SCoA + NADH. Succinyl-SCoA + GDP + P_i ⇌ succinate + GTP + CoASH GTP + ADP ⇌ GDP + ATP Succinyl-S-CoA + ADP + P_i ⇌ succinate + ATP + CoASH	
Various kinases (ATP: donor phosphotransferases)	ATP + acetate ⇌ acetyl phosphate + ADP (+H_2O)	
	ATP + creatine ⇌ creatine phosphate + ADP (+H_2O)	
Acyl transferases (e.g. phospho-transacetylase)	Acetyl phosphate + HSCoA ⇌ acetyl-SCoA + P_i	
Various synthetases (X:R) ligases	ATP + L-glutamate + L-cysteine ⇌ γ-L-glutamyl-L-cysteine + ADP + P_i	
Nucleoside diphosphate kinases (ATP: nucleoside diphosphate phosphotransferase)	ATP + NDP ⇌ ADP + NTP	P_i.
Various nucleotidyl transferases	ATP + FMN ⇌ FAD + PPi	

(•) Fig(xxii) Donor Functions of ATP



(•) Fig (xxiii)

Class ATP acts as donor of	Formation of	Example.
I. Phosphate (phospho-transferases, 2.7-2.7.4)	A. Anhydride or equivalent ($\Delta G' \geq 0$ kcal/mole)	Acetate + ATP ⇌ acetyl phospha-tase + ADP
	B. Ester, amide or equivalent ($\Delta G' < 0$ kcal/mol)	D-glucose + ATP ⇌ D-glucose-6-P + ADP
II. Pyrophosphate (pyro-phosphotransferases, 2.7.6)	A. Acceptor pyrophosphate	ATP + D-ribose-5-P ⇌ AMP + PR-PP
III. Adenosine-5'-phosphate	A. Enzyme-bound acyl adenylate and transfer to acceptor (6.1.1, 6.2.1)	ATP + RCO_2^- + enz ⇌ [enz. RCO_2^-. AMP] + PPi
		[enz. R-C(=O)-AMP] + CoASH ⇌ R-C(=O)-SCoA + AMP + enz
	B. Dinucleotide coenzymes (2.7.7)	ATP + FMN ⇌ FAD + PPi
	C. Polyadenylate (2.7.7)	(nATP) ⇌ $(AMP)_n$ + n PPi
IV. Adenosine	S-adenosyl methionine (2.4.2.1.a)	ATP + methionine ⇌ S-adenosyl methionine + trimetaphosphate
V. Driving force for reaction	Synthetases / Ligases (class 6)	

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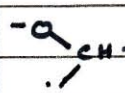
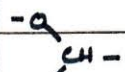
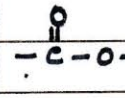
Table (continued.....)

Reaction or reaction type	Example of stoichiometry	Nature of R [gr] (fig xx)
Various ligases (synthetases of groups 6.1 and 6.2, first step)	ATP + enz + RCO_2^- ⇌ [enz·R–C(=O)–O–AMP]	[enz·R–C(=O)–O–]
Various ligases (second step)	[enz·R–C(=O)–O–AMP] + HX ⇌ AMP + R–C(=O)–O–X	[enz·R–C(=O)–O–]
ATP : D-ribose-5-phosphate pyrophospho transferase	ATP + D-ribose-5-phosphate ⇌ AMP + 5phospho-α-D-ribosylpyrophosphate (PRPP)	
Various NMP pyrophosphorylases, pentosyltransferases	PRPP + orotate ⇌ orotidine-5′-phosphate + PP_i	
Various ligases (gr 6.3)	ATP + L-pantoate + β-alanine ⇌ L-pantothenate + AMP + PP_i	
	ATP + xanthosine-5′-phosphate + NH_3 ⇌ GMP + AMP + PP_i	

This Work Done By

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Diseases -- Their Mini Definitions

&

Blood Grouping.

Sasoath Chakraborty.
B. Sc IIIrd Yr.
Roll- 17.

REFERENCES.

1. ; DOORLAND'S POCKET MEDICAL DICTIONARY :- : (26th Edition) Harcourt India : Saunders.

2. Talaro K.P and A. Talaro : (Third Edition : 1999) "Host defences" In "Foundation in Microbiology" (PP 392-395). WCB-Mc Graw Hill (Toronto).

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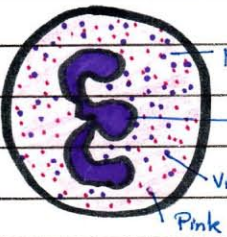
ADDITIONAL INFORMATION

I. Blood Staining :-

Diseases & Their Mini Definitions.



Neutrophil.

Increase in number of neutrophils ⟹ ① SEPTIC ENDOCARDITIS ② PUS FORMATION

• SEPTIC ENDOCARDITIS → (-kahr'-di-tis)

→ exudative and proliferative inflammatory alteration of the endocardium,

→ Usually characterized by the presence of vegetations on the surface of the endocardium (within the heart) or in the endocardium itself. and most commonly involving heart valve, but also affecting the inner lining of the cardiac chambers or the endocardium elsewhere.

→ **Causal organisms :-** Streptococci, Staphylococci, Enterococci Gonococci & Gram negative bacilli.

• PUS FORMATION :→

→ a protein rich liquid inflammation product made up of cells. (leukocytes) a thin fluid (liquour puris) & cellular debri.

→ **Causal organism:-** Strept pyogenes, Staph aureus etc.

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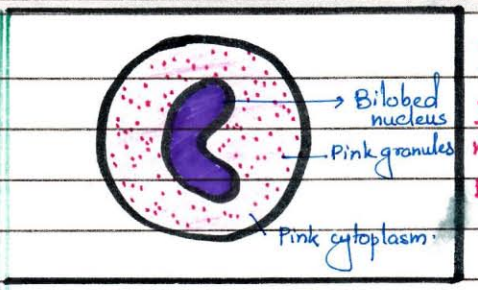


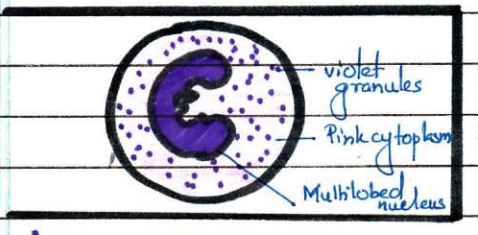
Eosinophils

Increase in number of Eosinophils ⟹ SCARLET FEVER.

- **SCARLET FEVER :→**

→ An acute disease caused by Group A β-hemolytic streptococci, marked by pharyngotonsillitis and a skin rash caused by an erythrogenic toxin produced by the organism

→ The rash is a diffuse, bright red erythema and desquamation of the skin begins as fine scaling with eventual peeling of the palms and soles.

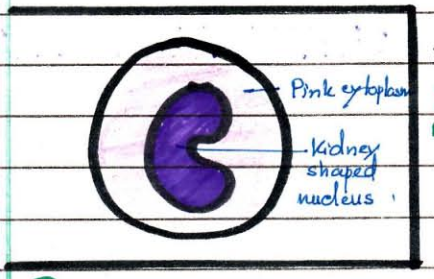


Basophils.

Increase in no of Basophils → BASOPHILIA
→ VIRAL INFECTION
→ LEUKOPENIA.

- **LEUKOPENIA. :→.**

Reduction in the number of leukocytes in the blood below about 5000 per cubic m.m. Basophilic Leukopenia pertains to Basophilia



Monocytes.

Increase in no of Monocytes → RICKETSIAL DISEASE
→ ROCKY MOUNTAIN SPOTTED FEVER.

- **RICKETSIAL DISEASE :-** Caused by Rickettsia.

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ROCKY MOUNTAIN SPOTTED FEVER

→ Infection with Rickettsia rickettsii

→ Transmitted by ticks, marked by fever, muscle pain, & weakness followed by a macular petechial (red spot due to escape of a small amount of blood) eruption that begins on the hands & feet & spreads to the trunk and face with other symptoms in the CNS & elsewhere.

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BLOOD GROUPING

THE BASIS OF HUMAN ABO ISOANTIGENS AND BLOOD TYPES.

→ The existence of human blood types was first demonstrated by an Austrian pathologist, **Karl Landsteiner in 1904**

→ While studying incompatibilities in blood transfusions, he found that the serum of one person could clump the red blood cells of another.

→ Landsteiner identified four distinct types, subsequently called the **ABO** blood groups.

→ Like the MHC antigens on White Blood cells, the ABO isoantigen markers on red blood cells are genetically determined & composed of glycoproteins.

→ These ABO antigens are inherited as two (one from each parent) of three alternative alleles * **A, B or O.**

→ **A & B** alleles are dominant over **O** and codominant with one another.

→ This mode of inheritence gives rise to four blood types (phenotypes), depending on the particular combination of genes.

→ Thus a person with an **AA or AO** genotype has **type A** blood; genotype **BB or BO** gives **type B**; genotype **AB** produces **type AB**; and genotype **OO** produces **Type O.**

IMPORTANT POINTS ABOUT BLOOD TYPES.

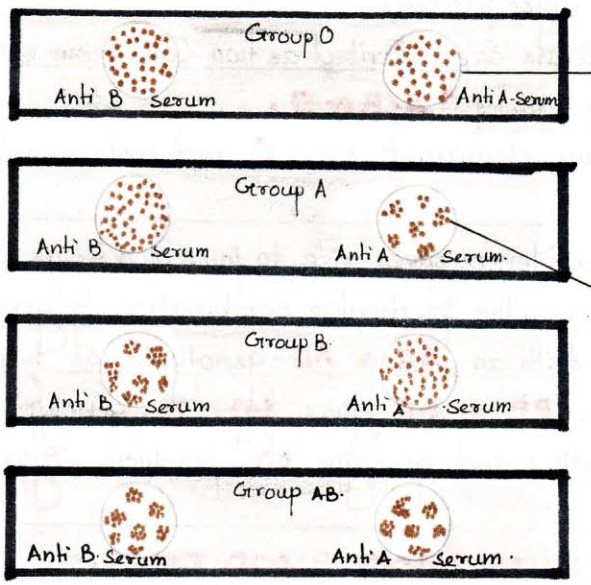
(1) They are named for the dominant antigen(s)

(2) The RBC's of type O persons have antigens, but not A & B antigens.

TABLE-1 CHARACTERISTICS OF ABO BLOOD GROUPS.

Genotype	Phenotype A & B* RBC Antigen	Prevalence in Population**	Serum Content of Antibodies.
OO	Neither	Most common	Both anti a & anti-b.
AA, AO	A	Second most common	Anti-b.
BB, BO	B	Third most common	Anti-a
AB	AB	Least common	Neither antibody

Legend: * Capital letters generally denote antigen; lowercase denotes antibody.
** True of most large population of mixed racial & ethnic groups.



Legend: Red blood cells (erythrocytes) of the type indicated at the top of each slide are mixed with blood serum of the type indicated below each reaction mixture (circle)

→ A clumped pattern of cells within a circle indicates that agglutination occurs.

FIG 1. AGGLUTINATION REACTIONS CONTROLLED BY THE ABO BLOOD TYPE LOCUS IN HUMANS :-

(3) Tissues other than RBC's carry A & B antigens.

GENETIC BASIS :- ABO BLOOD TYPE ALLELES IN HUMANS :-

Table 2 :- Genotypes & the corresponding Phenotypes (Blood Gr. Types) for the ABO Locus (Human)

GENOTYPE	PHENOTYPE.
$I^A I^A$ a $I^A I^O$	A
$I^B I^B$ a $I^B I^O$	B
$I^A I^B$	AB
$I^O I^O$	O

→ One of the most firmly established series of multiple alleles in humans involves the genetic locus controlling the blood types **A, B, AB & O.**

→ The ABO locus has three common alleles **I^A, I^B, & I^O**

→ I^A & I^B are **codominant** ($I^A I^B$ heterozygotes have both A & B antigens on their RBC's) & I^O is **recessive** ($I^O I^O$ homozygotes have no ABO antigens on their RBC's; $I^A I^O$ & $I^B I^O$ heterozygotes have A & B antigens, respectively, on their RBC's.

→ The ABO locus controls the type of glycolipids found on the surface of erythrocytes, apparently by specifying the type of **glycosyl transferases** (enzymes catalyzing the synthesis of polysaccharides) synthesized in the RBC's.

→ The specific types of glycolipids on the red cell surface provide the antigenic determinants that react with specific antibodies present in the blood serum.

→ Humans, like all other mammals, produce antibodies & circulate them in the blood serum as a defence mechanism against foreign substances.

Fortunately, no antibodies are synthesized (in normal individuals)

TABLE:- 3.

BLOOD TRANSFUSION COMPATIBILITIES FOR THE ABO BLOOD GROUPS.

BLOOD GROUP	TERMINAL SUGARS OF AGS PRESENT	ANTIBODIES PRESENT	RED CELL TYPES AGGLUTINATED	TRANSFUSIONS ACCEPTED FROM.
A	A (galactosamine)	Anti- B	B, AB	A or O
B	B (galactose)	Anti- A	A, AB	B or O
AB	A (galactosamine)	None	None	A, B, AB or O
O	None	Anti-A & Anti-B	A, B and AB	O.

Fig: 2.

INTERPRETATION OF BLOOD TYPING.

Legend : In this test, a drop of blood is mixed with a specially prepared antiserum known to contain antibodies against the A, B & RH antigens.

Fig ⇒ If that particular ag is not present, the R.B.C's in that droplet do not agglutinate & form an even suspension.

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that react with antigens present on the individual's own cells. However, when type A blood & type B blood are mixed, the anti A antibodies in the type B blood serum react with the antigens on the type A blood cells, & vice versa, which produces agglutination a clumping of cells [fig (v)]

→ Cross-matching blood types to determine compatibility is thus essential in blood transfusions.

→ In this process, blood donors and recepients are tested for the presence of antigens & antibodies that are incompatible.

→ Table (iii) summarizes the cell surface antigenic determinants & the serum antibodies present in the four major ABO blood types.

TRANSFUSIONS. → Individuals with blood type AB have both A & B antigens on their erythrocytes, but no anti A & B antibodies in their blood serum.

→ Type O individuals lack both ags, but carry both anti-A & anti-B abs in their blood serum

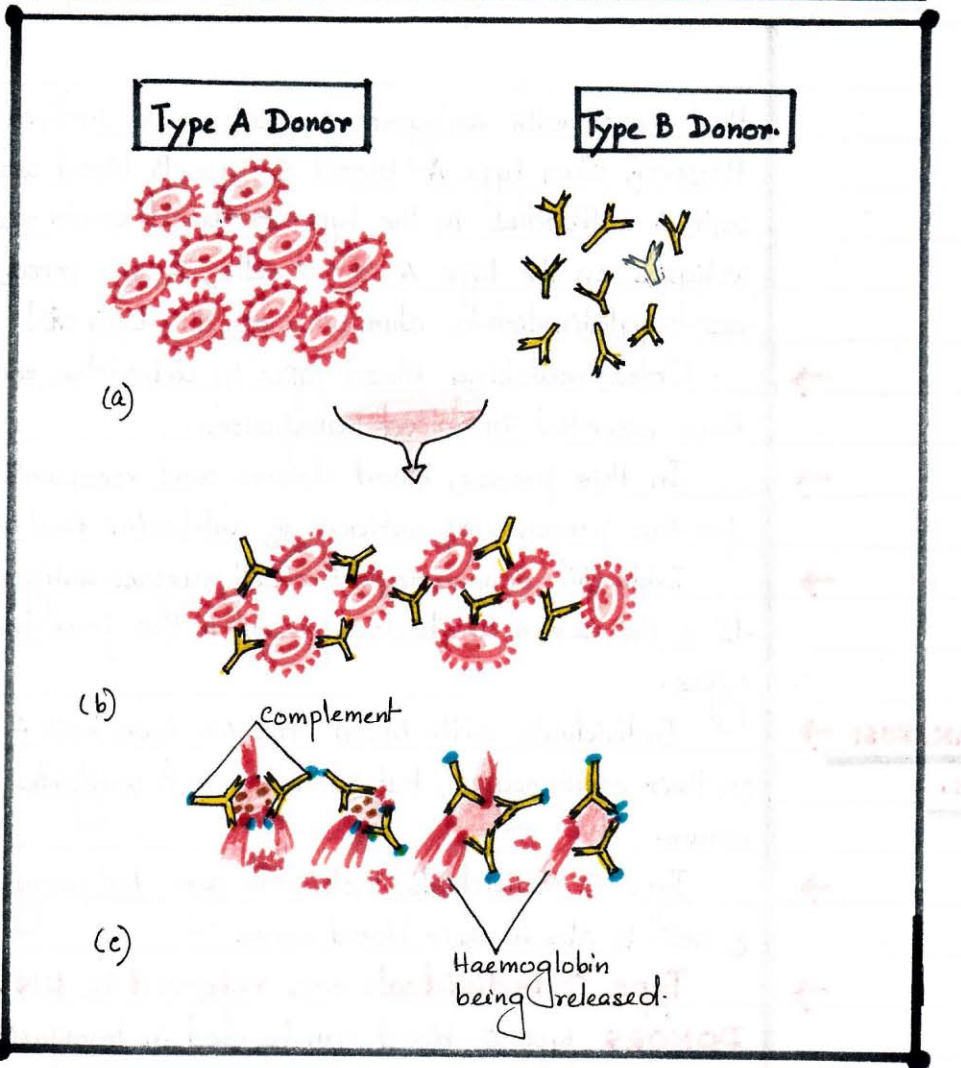
→ **Type O individuals are referred as UNIVERSAL DONORS**, type O blood can be used in transfusion for individuals of any blood type if the blood is introduced slowly enough to permit sufficient dilution of the Anti-A & Anti-B abs present in the serum of the donor.

→ Type AB persons are consequently called **UNIVERSAL RECEPIENTS.**

DEGREES OF ADVERSE REACTIONS IN TRANSFUSIONS.

Transfusion of the wrong blood type causes various degrees of adverse reaction.

Fig: 3 : MICROSCOPIC VIEW OF A TRANSFUSION REACTION



Legend :- (a) Incompatible blood. The red blood cells of the type A donor contain ag A, while the serum of the type B recipient contains anti-A abs that can agglutinate donor cells.

(b) Agglutination particles can block the circulation in vital organs.

(c) Activation of the complement by ab on the RBC's can cause haemolysis & anaemia. This sort of incorrect transfusion is very rare because of the great care taken by blood banks to ensure a correct match.

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→ The severest reaction is massive hemolysis when the donated red blood cells react with recipient antibody & trigger the com-plement cascade (fig. 3).

→ The resultant destruction of red cells leads to systemic shock & kidney failure brought on by the blockage of glomeruli (blood filtering apparatus) by cell debris.

→ Death is a common outcome.

Other reactions caused by RBC destruction are **fever, anemia & jaundice**

→ A transfusion reaction is managed by immediately halting the transfusion, administering drugs to remove hemoglobin from the blood, and beginning another transfusion with RBC's of the correct type.

A CLOSER APPROACH.
MICROFILE: THE ORIGIN OF ABO ANTIGENS.

→ **The A and B genes** each code for an enzyme that adds a terminal carbohydrate to RBC receptors during maturation. **RBC's of Type A** contains an enzyme that adds **N-acetyl glucosamine** to the receptor; RBC's of **type B** have an enzyme that adds **D-galactose**; RBC's of **type AB** contain both enzymes that add both carbohydrates; and RBC's of **type O** lack the genes & enzymes to add a terminal molecule.

→ The genetics of ABO ags were once used to rule out paternity. For eg if a man is type A, the mother type O, & the child type B, we know this man could not have fathered this child.

→ However this same logic cannot prove paternity. If the child is type A instead, it is this same logic for the man to be the

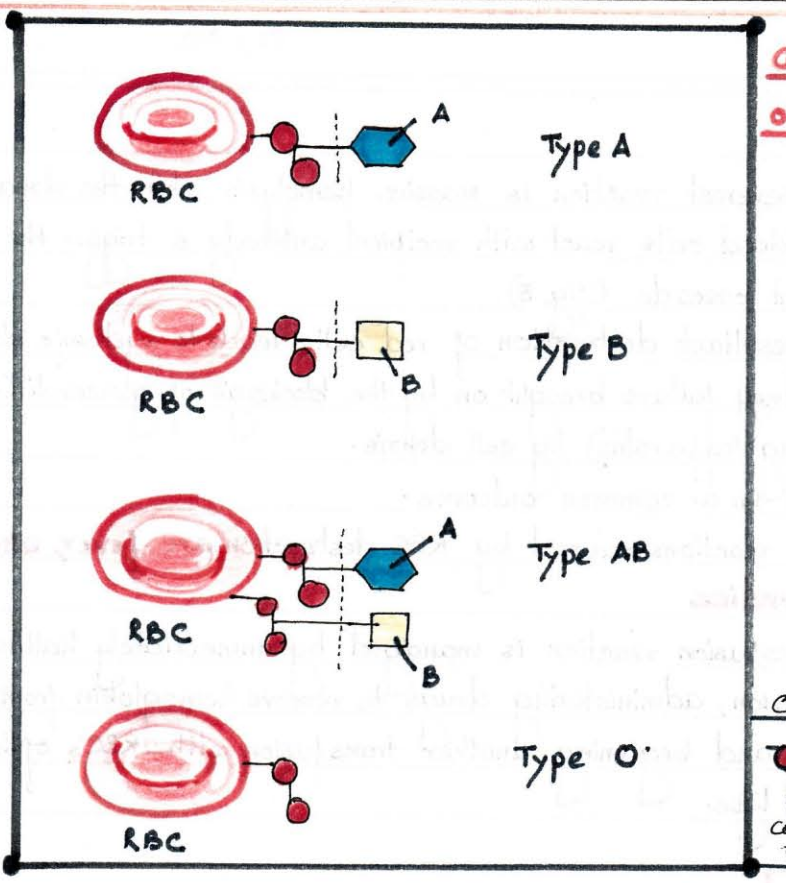
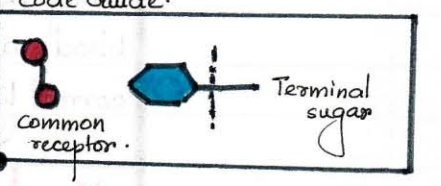


Fig 4:-
GENETIC BASIS FOR AB AGS. ON RBC.



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father, but so could some other man with blood type A.

→ Highly sensitive methods based on specific & variable MHC ags & DNA fingerprinting have been developed to gather more precise evidence of paternity & maternity (in cases of kidnapping & adoption, for instance).

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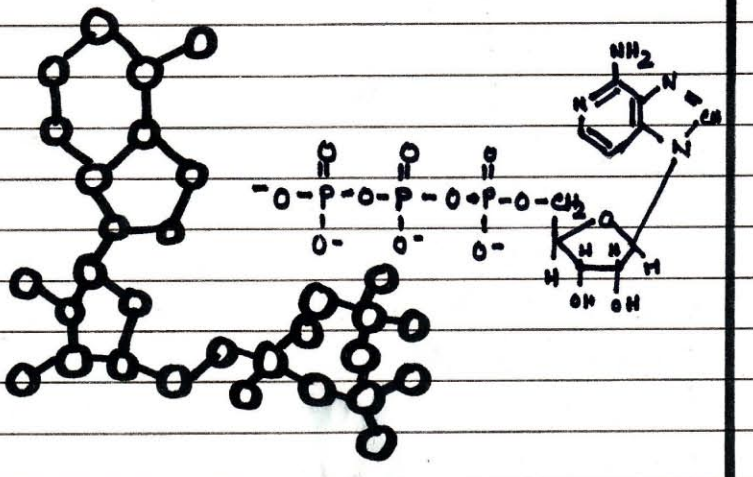
METABOLISM

ATP

(•) Name : Saswati Chakraborty
(•) Std : B.Sc III Yr
(•) Roll : 17



Adenosine Tri Phosphate.

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REFERENCES :-

1. A.L. Lehninger : (1995) "Bioenergetic Principles and the ATP cycle", Metabolic and Energy Transfer Pathways : A Survey", "Electron transport and oxidative Phosphorylation" IN FUNDAMENTALS OF BIOCHEMISTRY (PPs - 267 - 289 - 813, 865 - 895) PUB: SPARKS (MARYLAND).

2. Martin. W.D., Mayes A.P., and V.W Rodwell : (1985) "Bioenergetics" In Harper's Review of Biochemistry (PPs 64 - 69) PUB: MARUZEN ASIAN EDITION, Lange Medical Publications

3. Champe. P.C and R.A. Harvey : (1988) "ATP AS ENERGY CARRIER" IN LIPPINCOTT'S ILLUSTRATES REVIEWS : BIOCHEMISTRY (PPs : 99-105) Pub: (7th edition).

4. Todd. W.R., West E.S and H.S Mason (1985) "Introduction to Intermediary Metabolism" In TEXTBOOK OF BIOCHEMISTRY (PPs 850-851, 884-895)

5. Mahler. H.R and E.H. cordes : (1974) Aerobic Respiration IN BIOLOGICAL CHEMISTRY (2nd ed^{n}) (PP- 378-382) PUB: Harper and Row Publishers (New York) - [HARPER INTERNATIONAL]. - [New York]

6. Conn. E.E and P.K stump : (1974: 4th ed^{n}) Biological Energetics In OUTLINES OF BIOCHEMISTRY (PP- 142-148) Pub: WILEY EASTERN LTD (California).

7. D. E. Sadave : (1992). Energy Transduction : Mitochondria, In CELL BIOLOGY (organelle-structure function) (PPs - 110 - 114)
Pub : Jones and Bartlett Publishers (California).

8. PELCZAR . J.M., CHAN. E.C.S and N.R.KRIEG : (1993) Microbial Metabolism In MICROBIOLOGY (PP - 171-196)
Pub : Tata Mc Graw Hill Publishing Co Ltd. (Delhi).

9. A. J. SALLE : (1992) Fermentation of Carbohydrates In FUNDAMENTAL PRINCIPLES OF BACTERIOLOGY (PP - 475-505)
Pub : Tata Mc Graw Hill Publishing Co Ltd (Delhi).

10. Power. C.B and H.F Daginawala : (1991) Carbohydrate Metabolism In GENERAL MICROBIOLOGY. (PP - 488)
Pub : Himalayan Publishers (Mumbai).

11. Duodoroff Michael : (7th Edition) ATP, Metabolism . In ENCYCLOPEDIA OF SCINCE AND TECHNOLOGY (Vol I and II) (PP 120 - 121, 4 - 5)
Pub : Tata Mc Graw Hill Publishing Co Ltd (Delhi)

12. C. J. Avers : (1992) Aerobic Respiration In MOLECULAR CELL BIOLOGY. (PP - 328 - 329).
Pub :

13. L. STRYER : (1987) "ATP" In BIOCHEMISTRY (3rd edition)
Pub : H. Freeman and company (PP. 950, 953, 415, 416).

14. H. G. Schlegel : (1995) Modes of Energy Yielding Metabolism In "GENERAL MICROBIOLOGY" (PP : 343). Pub : Cambridge University Press.

15. Website : www.google.com.

16. Devlin. R. M and F. H. Witham : (1997) Energy Liberation and Utilization In. PLANT PHYSIOLOGY. (PP-318 - 319). Pub: CBS Publishers & Distributors (Delhi).

17. Dubey. R. C and D. K Maheshawari : (2000) ATP In GENERAL MICROBIOLOGY (2nd Ed") (PP-267 - 269) Pub : S. Chand and company Ltd.

18. Roleries., E. D. P and B. M. F De. Roberts Jr : (1997) ATP IN CELL AND MOLECULAR BIOLOGY (PP-26) Pub : B. I. Waverly Co Ltd.

19. M. Swaminathan : (1981) ATP in BIOCHEMISTRY OF STUDENT (P.P- 461-532) Pub : Geeta Book House (Mysore).

20. D. Friefelder : [2nd edition 1994] ATP IN MOLECULAR BIOLOGY (PP - 30-36) Pub : Narasa Publications House.

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METABOLISM

AN OVERVIEW

- Definitions of metabolism.
- Catabolism and Anabolism.
- Classification of micro-organisms on the basis of energy & carbon sources.
- Bioenergetics.
- Coupling through ATP and through pyridine nucleotides.

INTRODUCTION : [Definition].

— The term metabolism denotes all the organized chemical activities performed by a cell, which comprise two general types :

→ energy production

→ energy utilization

— The term intermediary metabolism is a rather incomplete definition which only highlights by eliciting as the sum total of all the enzyma-tic reactions occurring in the cell.

— Four specific functions of metabolism are :-

→ (1) To extract chemical energy from the environment, either from organic nutrients or from sunlight.

→ (2) To convert exogenous nutrients into the building blocks or precursors of the macromolecular components of cells.

→ (3) To assemble the building blocks into proteins, nucleic acids, lipids.

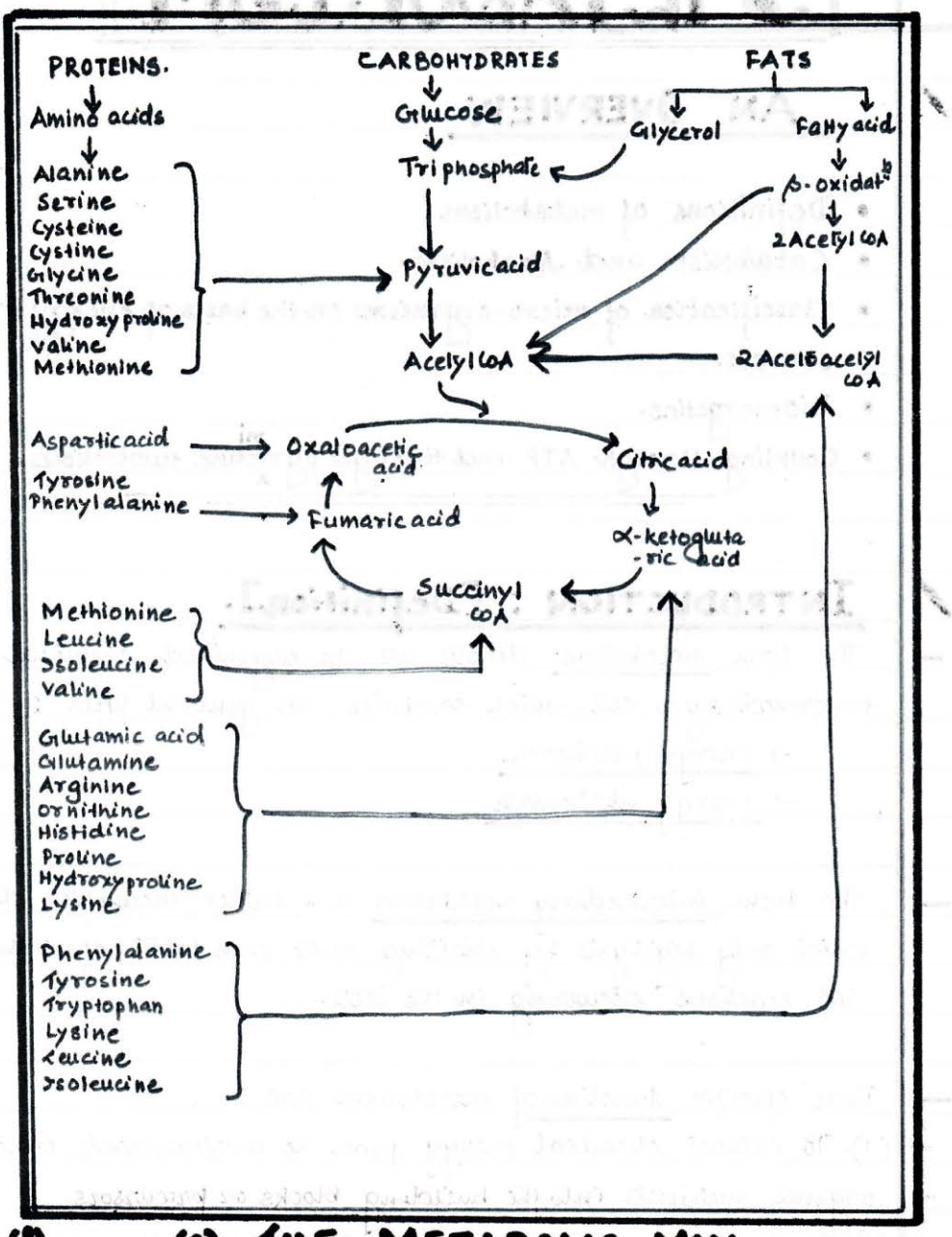

Fig: 4) (*) THE METABOLIC MILL

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and other characteristic cell components and

→ (4) To form and degrade those biomolecules required in specialized functions of cells.

TYPES OF METABOLIC REACTIONS.

METABOLISM

CATABOLISM.

(•) It is the enzymatic degradation, largely by oxidative reactions, of relatively large nutrient molecules (carbohydrates, lipids & proteins) coming either from the environment of the cell or from its own nutrient storage depots into a series of smaller, simpler molecules e.g lactic acid, acetic acid, CO_2, ammonia a, urea.

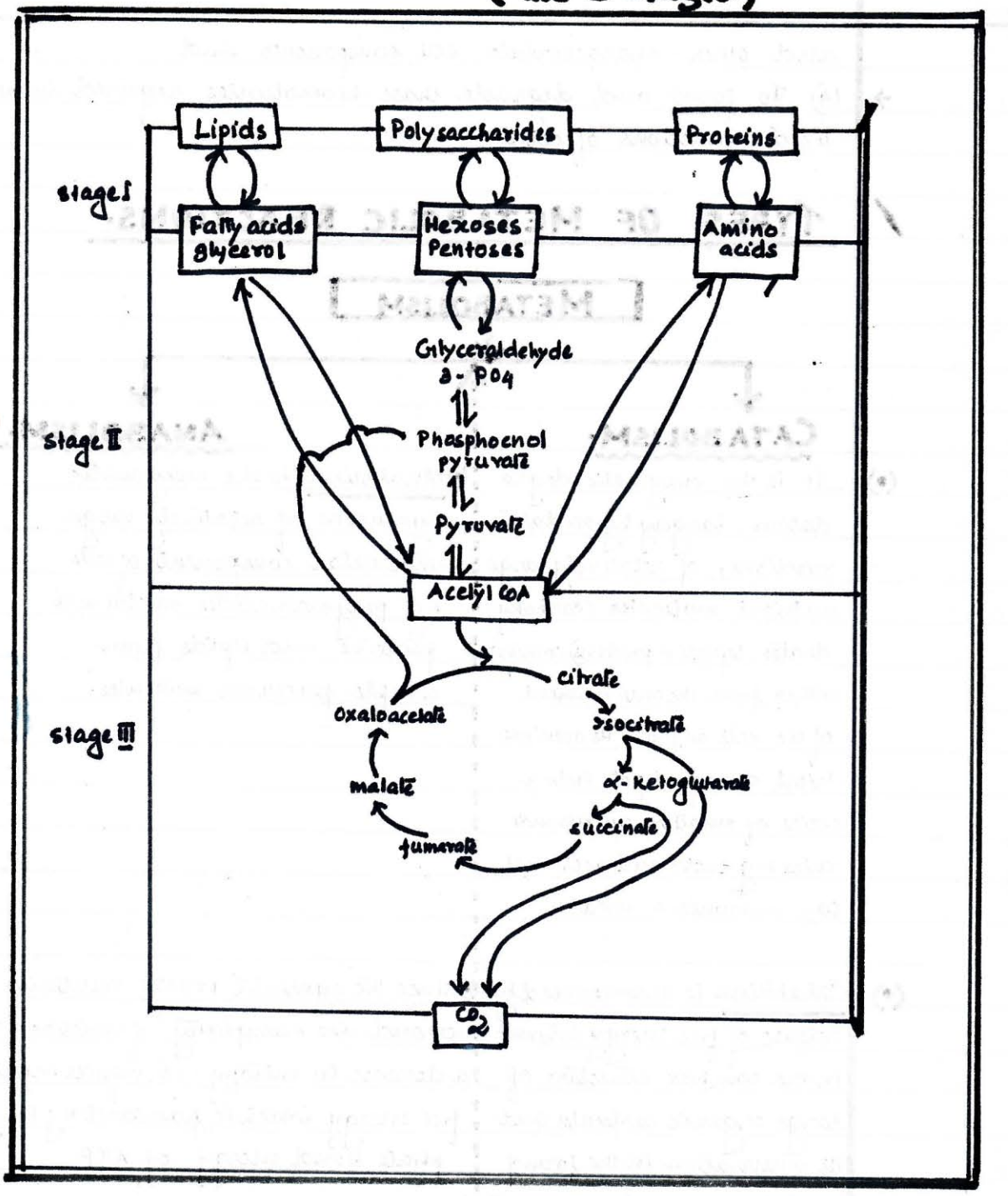
(•) Catabolism is accompanied by release of free energy inherent in the complex structure of large organic molecules and its conservation in the form of the phosphate bond energy of ATP.

ANABOLISM.

(•) Anabolism is the enzymatic synthesis of relatively large molecular components of cells e.g polysaccharides, nucleic acids proteins and lipids from simple precursor molecules.

(•) Since the synthetic process results in increased size & complexity of structure & thus a decrease in entropy, it requires input of free energy which is furnished by the phosphate bond energy of ATP.

Fig2: (*) CATABOLISM, ANABOLISM, AMPHIBOLISM
(The 3 stages.)



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AMPHIBOLIC PATHWAY :-

Although the pathways of catabolism and anabolism are not identical the stage III [fig 2] constitutes a central meeting ground or pathway which is accessible to both. This central route, is called an amphibolic pathway. (amphi → dual).

CLASSIFICATION OF M.O.S ON THE BASIS OF ENERGY CARBON SOURCES :

•> Division on the basis of utilization of carbon source.

- AUTOTROPHS
  - Autos : self
  - trophos : feeders
  - → Photo autotrophs
  - → Chemolithotrophs
- Heterotrophs
  - Hetero : other
  - trophos : feeders

•> Division on the basis of energy source

- Phototrophs
  - Photo organo trophs
  - Photolitho trops.
- Chemotrophs
  - Chemoorgano trophs
  - Chemo lithotrophs.

•> Division on the basis of oxidizing agent for nutrient breakdown.

- (Aerobes) [those which grow in presence of O_2]
- (Anaerobes) [those which grow in absence of O_2]

Fig 3 (*) The increase of entropy :

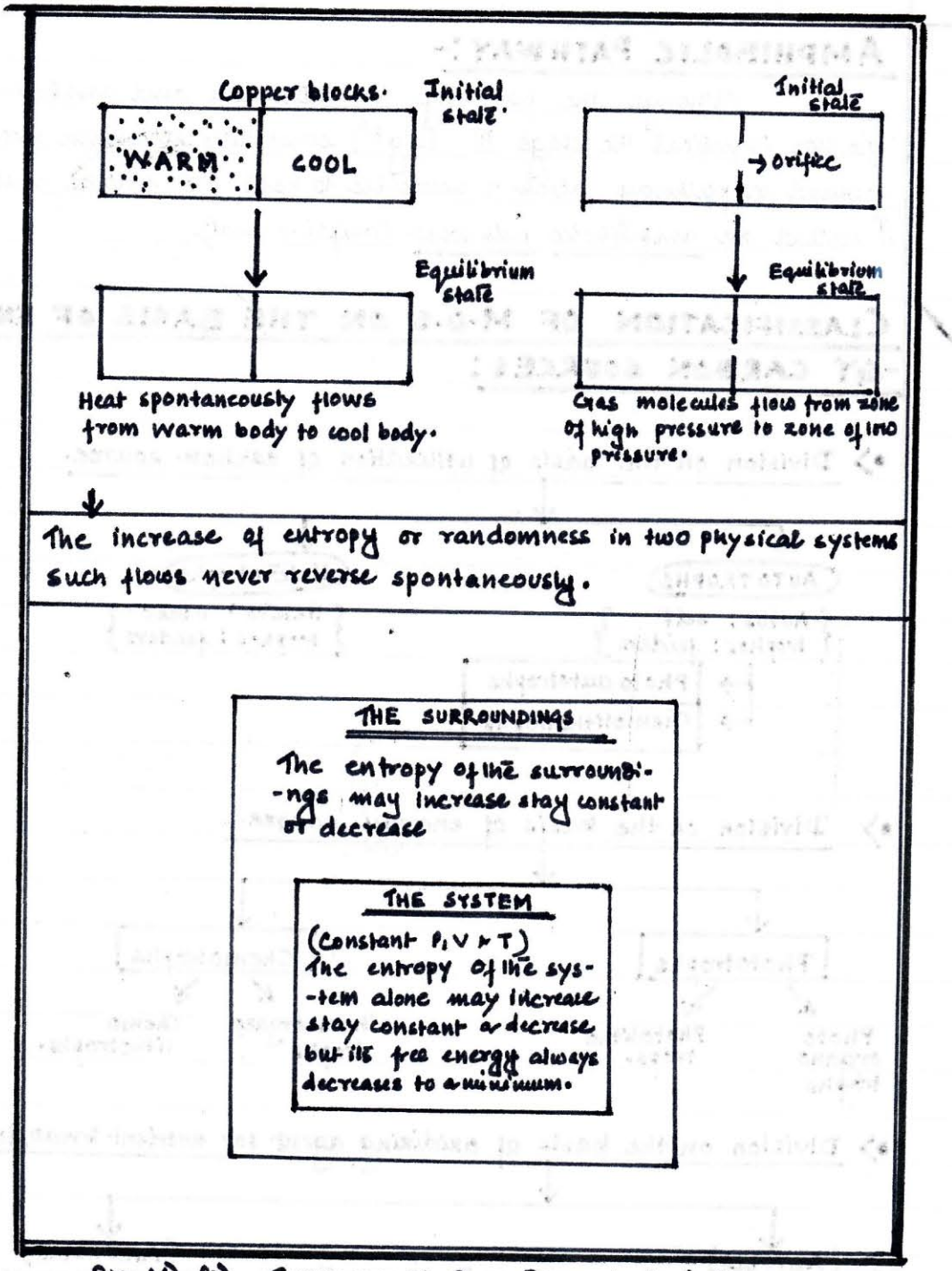

Fig (4) (*) Summary Of Free Energy And Entropy.

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BIOENERGETICS:

(TERMINOLOGIES INVOLVED).

(•) **ENTROPY** :- Entropy is defined (for the moment) as the degree of disorder or randomness. ['S']

(•) **EQUILIBRIUM** :- An Equilibrium is defined as a state in which no further net chemical or physical change is taking place and in which temperature, pressure and concentration are uniform throughout the system.

∴ All "real" processes occurring in our physical world including the process of life are Irreversible ∴

(•) **FREE ENERGY** :- Entropy changes during chemical reactions are not always easily measured or calculated. However the change in entropy during a process is quantitatively related to changes in total energy of the system by a third function called the Free Energy. [ΔG]

(•) **ENTHALPY** :- The change in function is known as enthalpy

(•) **IMPORTANT EQUATIONS**:

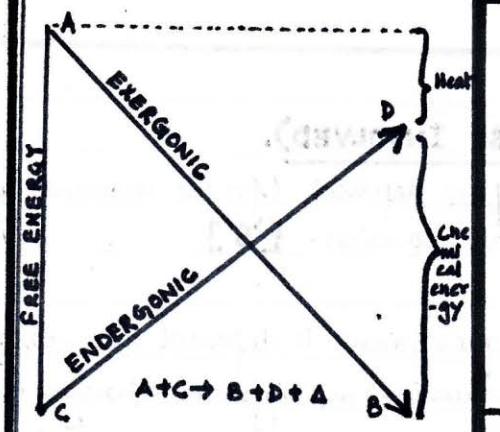
(1) $\Delta G = \Delta H - T\Delta S$

(2) $\Delta H = \Delta E + \Delta PV$

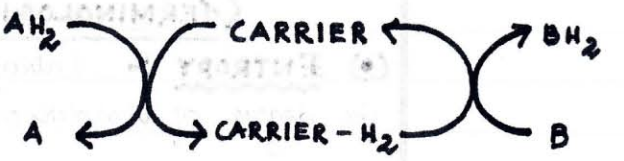
(3) $\Delta G = \Delta E + T\Delta S$

(4) $\Delta E = \Delta G + T\Delta S$

(•) "COUPLING - REACTION"



Coupling of an exergonic to an endergonic reaction. → Fig (6)(•)



Coupling of dehydrogenation and hydrogenation reactions by an intermediate carrier → Fig (6)(•)

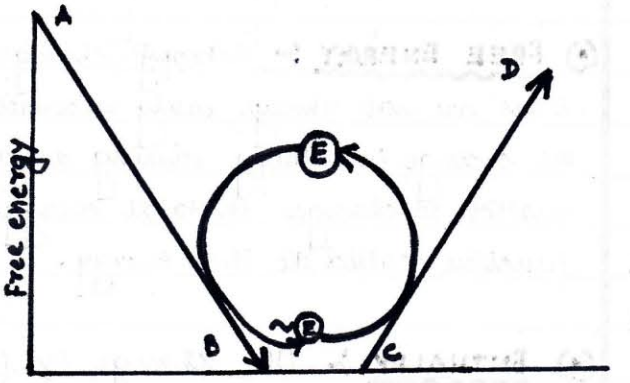
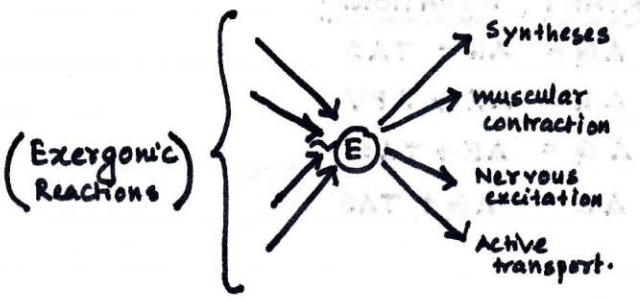


Fig (7) → Transference of free energy from an exergonic to an endergonic reaction through the formation of a high energy intermediate compound. (•)



(•) Fig (8) → Transduction of energy through a common high-energy compound to energy-requiring (endergonic) biologic processes.

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BIOENERGETICS OF COUPLED REACTION:-

Coupling – ATP & Pyridine Nucleotides.

→ First reaction in the glycolytic pathway.

[The phosphorylation of glucose to glucose 6-P which is highly endergonic and would not proceed as such under physiologic conditions]

(1) Glucose + P_i ⟶ Glucose 6-P + H_2O

($\Delta G^{0'} = +13.8$ KJ/mol)

(2) ATP ⟶ ADP + Pi ($\Delta G^{0} = -36.8$ KJ/mol)

Reaction couples with another reaction (hydrolysis of the terminal PO_4 of ATP) that is more exergonic.

(3) Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose 6-P + ADP ($\Delta G^{0'} = -23.8$ KJ/mol)

INTERCONVERSION OF ADENINE NUCLEOTIDES

The enzyme adenylate kinase (myokinase) is present in most cells. It catalyzes the interconversion of ATP & AMP on the one hand and ADP on the other.

(4) Adenosine – Ⓟ~Ⓟ~Ⓟ (ATP) + Adenosine – Ⓟ (AMP)

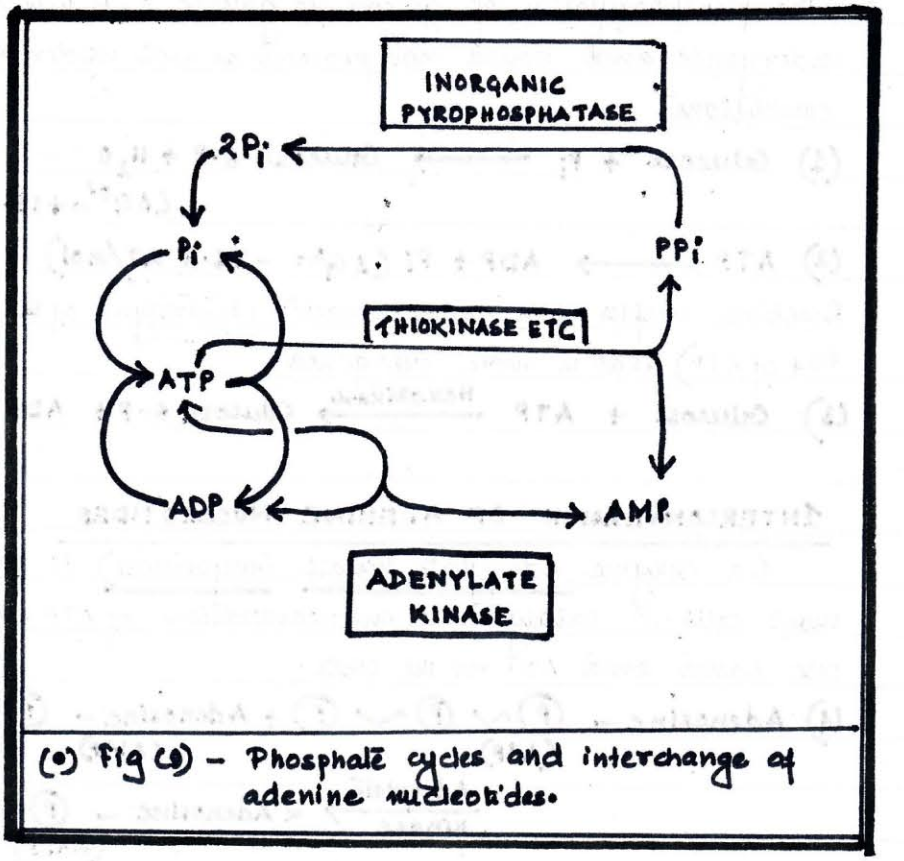
$\xrightarrow[\text{kinase}]{\text{Adenylate}}$ 2 Adenosine – Ⓟ~Ⓟ (2ADP)

When ATP reacts to form AMP, inorganic pyrophosphate (PP_i) is formed, as occurs – [activation of long chain fatty acids].

(5) ATP + CoA · SH + R · COOH $\xrightarrow{\text{Thiokinase}}$ AMP + PP_i + R · CO~S CoA

($\Delta G^{0'} \rightarrow -1.6$ Kcal/mol)

(6) $PP_i + H_2O \xrightarrow[\text{pyrophosphatase}]{\text{inorganic}}$ 2Pi



(•) Fig (9) – Phosphate cycles and interchange of adenine nucleotides.

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A combination of the above reactⁿs makes it possible for phosphate to be recycled and the adenine nucleotides to interchange (fig 9.)

Nucleoside Phosphates Related to ATP & ADP.

By means of the enzyme nucleoside diphosphate kinase, nucleosides triphosphates similar to ATP but containing a different base from adenine, can be synthesized from their diphosphates. e.g

{Nucleoside Diphosphate kinase}

ATP + UDP ⟷ ADP + UTP (uridine triphosphates)

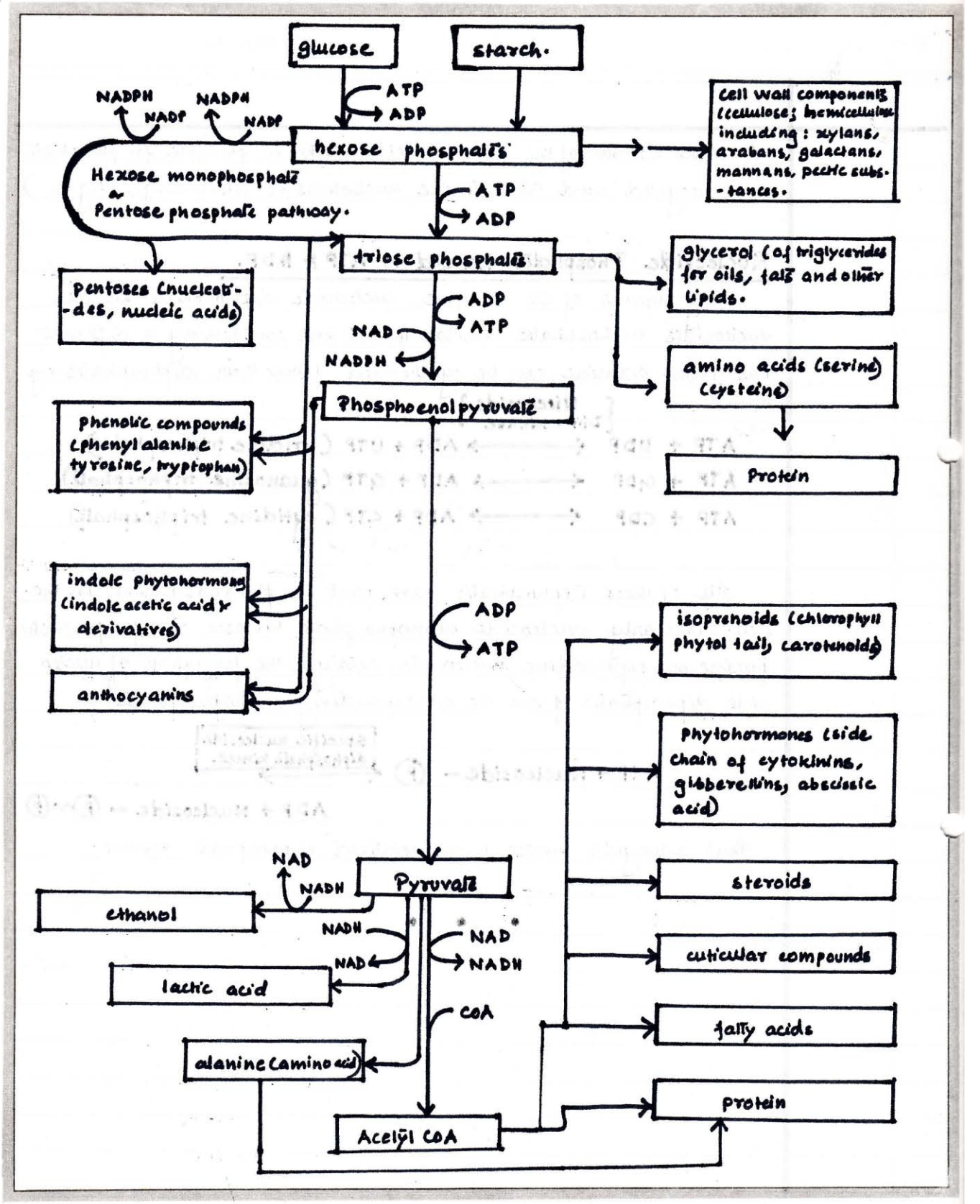
ATP + GDP ⟷ ADP + GTP (guanosine triphosphate)

ATP + CDP ⟷ ADP + CTP (cytidine triphosphate)

All of these triphosphates take part in phosphorylations in the cell. Similarly nucleoside monophosphate kinases, specific for each purine or pyrimidine nucleoside, catalyze the formation of nucleoside diphosphates from the corresponding monophosphates.

ATP + Nucleoside – Ⓟ ⟷ (specific nucleoside diphosphate kinase) ADP + Nucleoside – Ⓟ~Ⓟ

Thus adenylate kinase is a specialized diphosphate kinase.


glucose
starch.
ATP
ADP
NADPH
NADP
NADPH
NADP
hexose phosphates
cell wall components (cellulose; hemicelluloses including: xylans, arabans, galactans, mannans, pectic substances.
Hexose monophosphate & Pentose phosphate pathway.
ATP
ADP
pentoses (nucleotides, nucleic acids)
triose phosphates
glycerol (of triglycerides for oils, fats and other lipids.
ADP
ATP
NAD
NADH
amino acids (serine) (cysteine)
Phosphoenolpyruvate
Protein
phenolic compounds (phenylalanine tyrosine, tryptophan)
indole phytohormones (indole acetic acid & derivatives)
anthocyanins
ADP
ATP
isoprenoids (chlorophyll phytol tail, carotenoids)
phytohormones (side chain of cytokinins, gibberellins, abscissic acid)
NAD
NADH
Pyruvate
steroids
ethanol
NADH
NAD
NAD
NADH
cuticular compounds
lactic acid
CoA
fatty acids
alanine (amino acid)
protein
Acetyl CoA

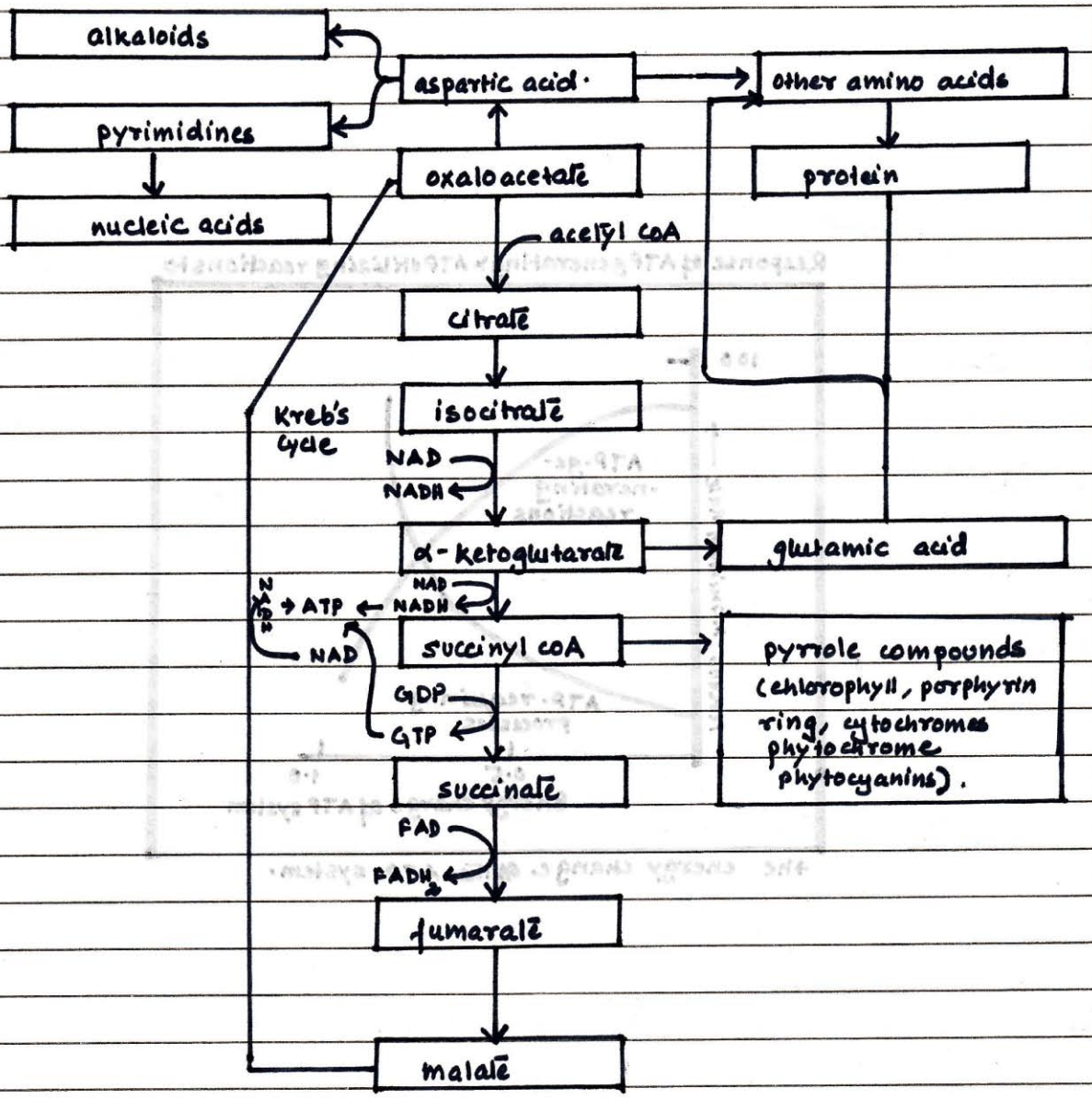
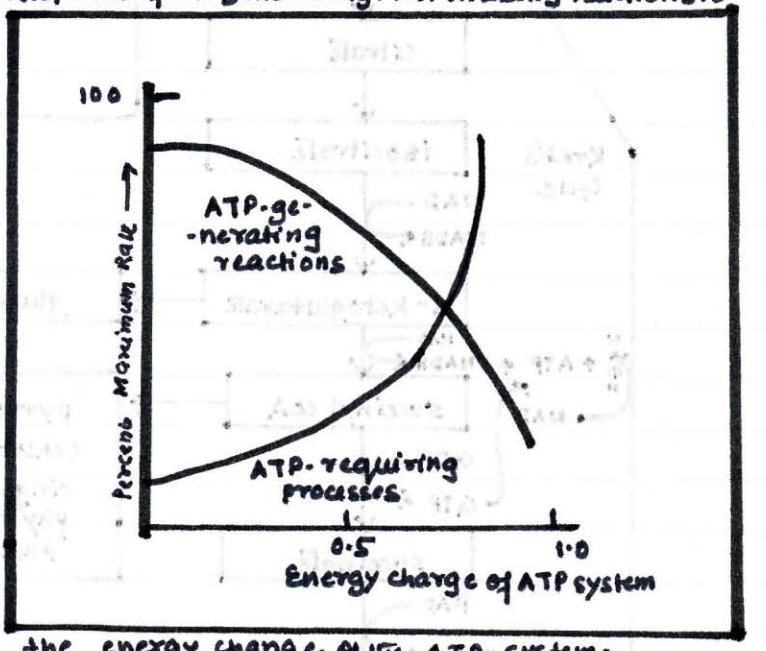


Fig aside x above :- Overview of Relationship of Cellular Components and Energy Yielding Reactions of Respiration --

"INTER METABOLIC RELATIONSHIPS"

Response of ATP generating & ATP utilizing reactions to

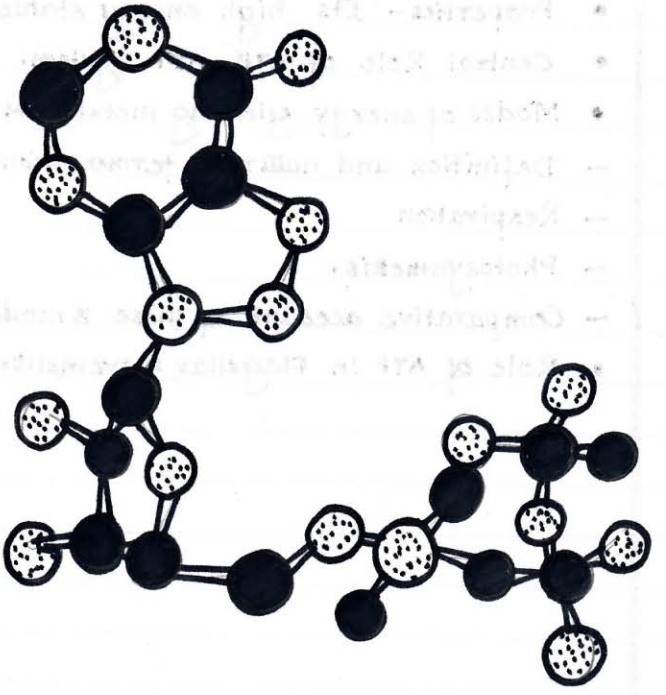


the energy change of the ATP system.

ATP - (Adenosine Triphosphate)

The Currency Of Cell....

Ball & Stick Model of Adenosine Triphosphate.



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ADENOSINE TRI-PHOSPHATE

"ATP"

(•) AN OVERVIEW :-

- Introduction
- Structure
- Properties - Its high energy status.
- Central Role of ATP - ADP system.
- Modes of energy yielding metabolism.
  - Definition and nature of fermentation
  - Respiration
  - Photosynthesis.
  - Comparative account of these 3 modes.
- Role of ATP in Flagellar movement.

ATP - The currency of cell

ATP - The energy carrier.

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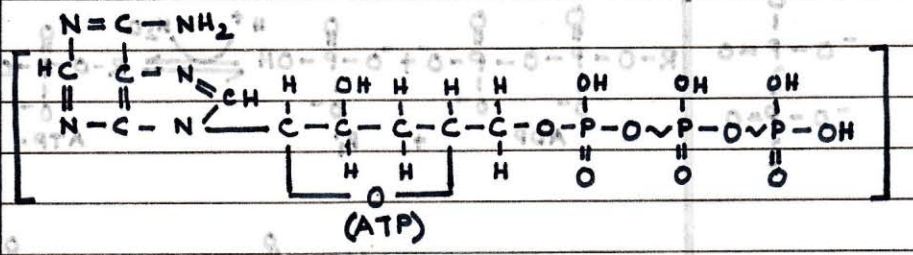
INTRODUCTION :

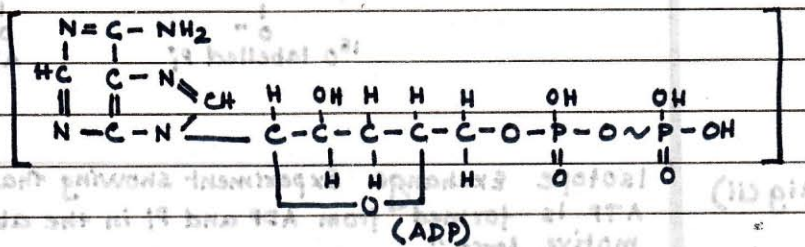
- ATP was first isolated from acid extracts of muscle in 1929 by Fiske and Subbarow.
- Its structure was deduced some years later by degradation experiments and ultimately confirmed by total chemical synthesis by Todd and his colleagues in 1948.
- From its first discovery ATP was suspected to play a role in cellular energy transfer, but it was not until 1939-1941 that Lipmann proposed it serves as a principal means of transfer of chemical energy in the cell.

STRUCTURE :

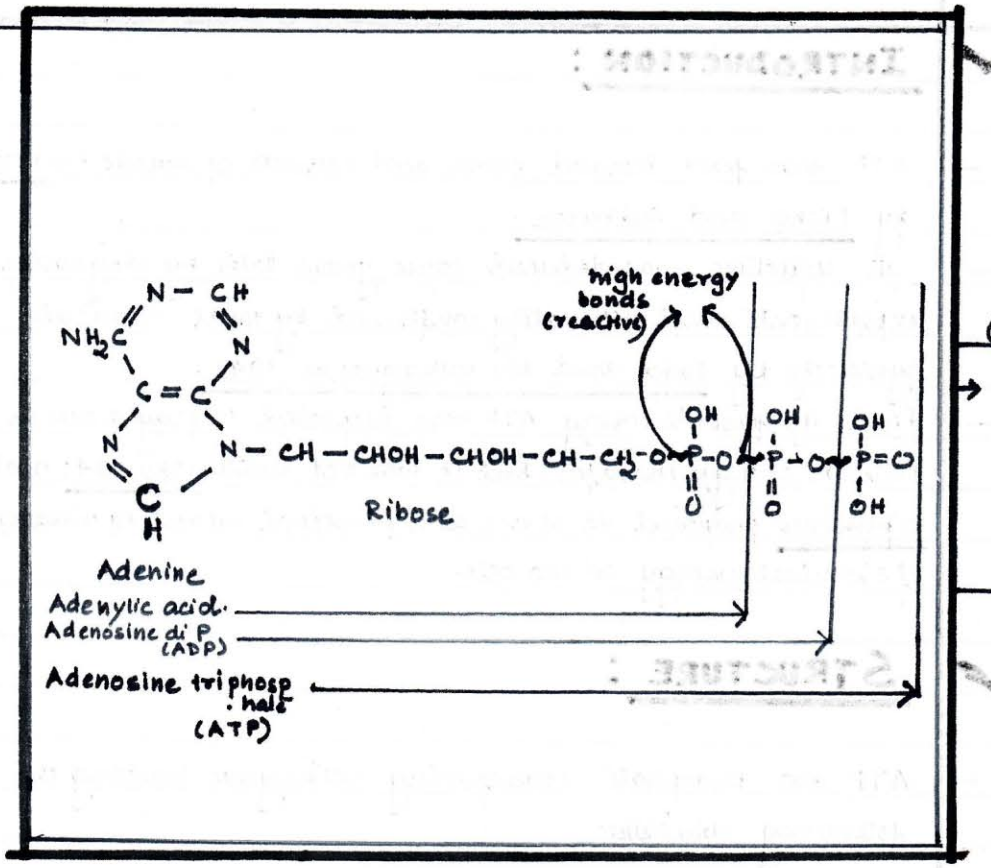
- ATP are phosphate-transferring coenzymes having the following structure.



(ATP)

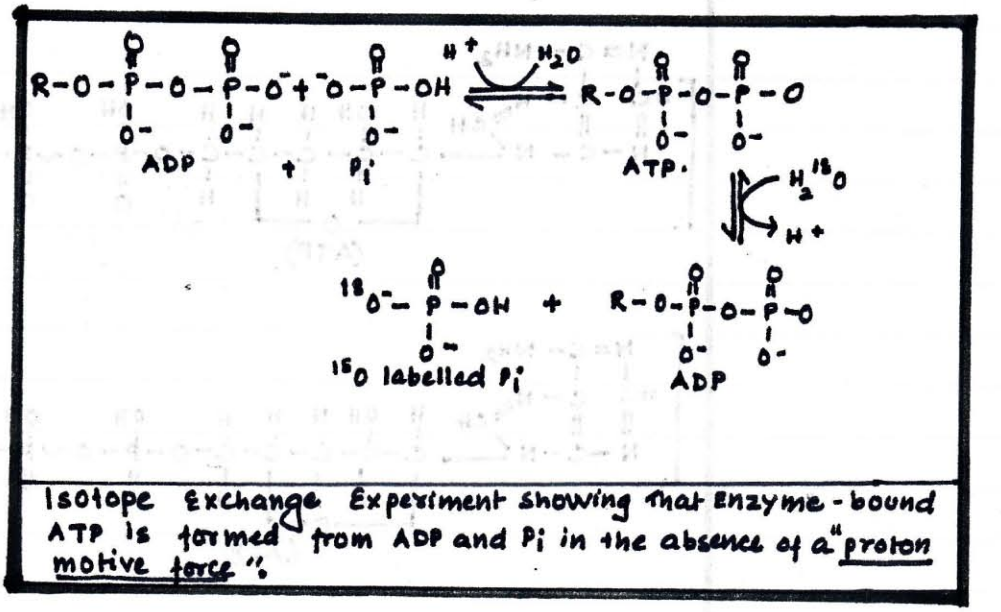


(ADP)



(*) Fig (i)

→ STRUCTURE OF ADENYLIC ACID AND PHOSPHATE DERIVATIVES ADP AND ATP.



(*) Fig (ii)

Isotope Exchange Experiment showing that Enzyme-bound ATP is formed from ADP and Pi in the absence of a "proton motive force".

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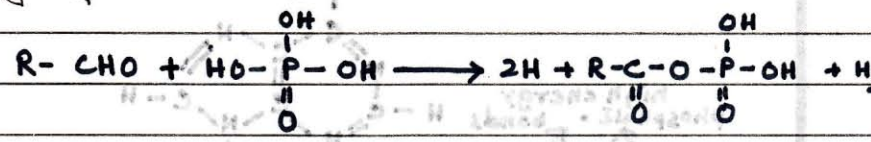
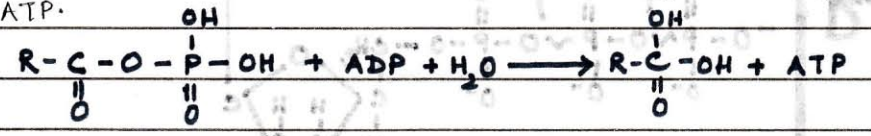
- ATP is the "universal fuel" of the living cell.
- It contains two high energy phosphate bonds (~) and each stores about 12,000 calories and releases about 7,500 calories when broken.
- ATP is produced by two series of reactions :-

(1) an aldehyde reacts with an inorganic phosphate to give hydrogen and an acid phosphate



(2) the acid phosphate reacts with ADP to give an organic acid & ATP.

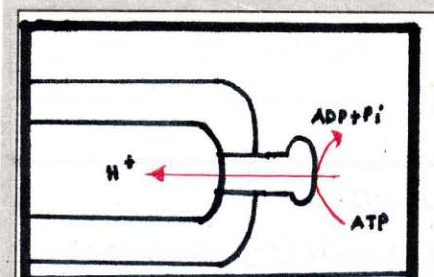


- ATP due to its high energy bonds and PO_4 groups is able to donate number of PO_4 groups to a number of metabolic linkages, thereby converting them to activated forms.
- Their increased free energy allows a phosphorylated intermediate to participate in biosynthetic reactions.
- The special reactivity of the high energy bonds of ATP is apparent when $\Delta G^{0\prime}$ (Free energy) of their hydrolysis is compared with the $\Delta G^{0\prime}$ of hydrolysis of the phosphate of AMP attached to adenosine by an ester linkage. Therefore less reactive and termed as low energy bonds.

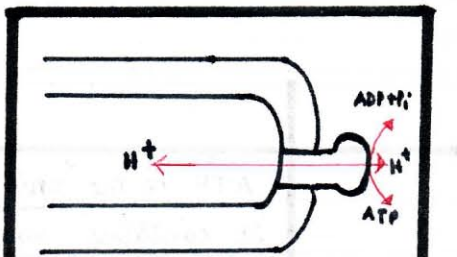
Adenosine – Ⓟ~Ⓟ~Ⓟ + H_2O → Adenosine – Ⓟ~Ⓟ + Ⓟ

ADP. $\Delta G^{0\prime} = -7.3$ Kcal.

The chemiosmotic theory of proton electrochemical coupling.

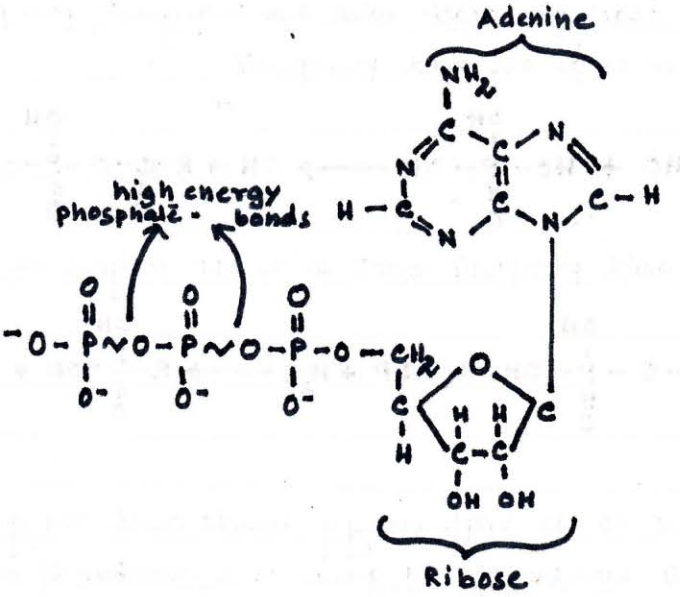


One proton pump: ATP hydrolysis drives protons across the pump. As H^+ builds up on the other side it sets up a potential gradient to drive ATP synthesis.



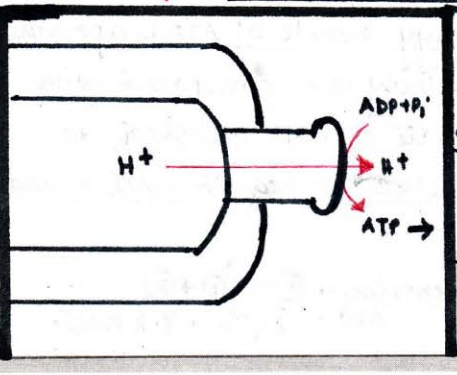
Equilibrium: between energy derived from ATP hydrolysis + energy needed to pump protons against an increasing gradient.

Coupling



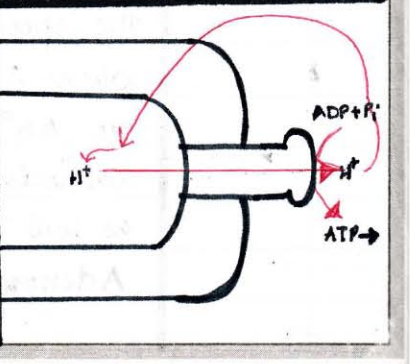
Coupling

• Fig (iii) — "ATP AS ENERGY CARRIER"



ATP removed as it is made the proton gradient drives ATP synthesis. This depletes the gradient, unless it is constantly replenished.

Second proton pump: This replenishes the proton gradient so that ATP synthesis can continue.



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Adenosine — Ⓟ ~ Ⓟ + H_2O → Adenosine — Ⓟ + Ⓟ

$\Delta G^{0\prime}_{AMP} = -7.3$ kcal.

PROPERTIES :

(1) Its high energy status : ATP as energy carrier.

A. Chemical reactions are coupled through common intermediates

- Two chemical reactions have a common intermediate when they occur sequentially so that the product of the first reaction is the substrate for the second.

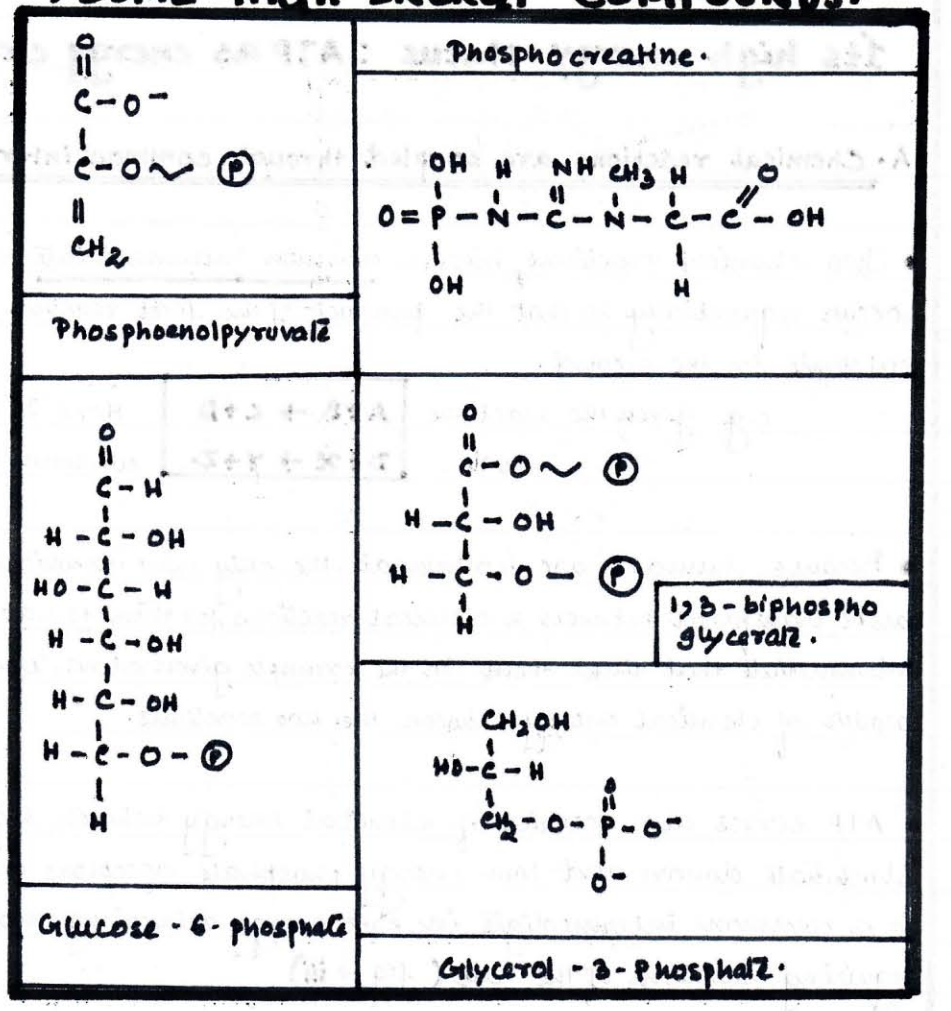
e.g. given the reactions $A + B \rightarrow C + D$ and $D + X \rightarrow Y + Z$ — Here 'D' is the common intermediate

- Because humans are isothermal, the only way in which energy can be transferred between 2 chemical reactions for them to have a common intermediate that links them. In the example given above, D could be a carrier of chemical energy between the two reactions.

- ATP serves as a carrier of chemical energy between high energy phosphate donors and low-energy phosphate acceptors because it is a common intermediate in both energy delivering and energy requiring reactions of the cell (fig → iii)

(Fig iv)

(e) :- SOME HIGH ENERGY COMPOUNDS :-



N·B → ENERGY CHANGE.

The relative amount of high energy forms of ATP (ATP, ADP) can be calculated using the following formula:

$$EC = \frac{1}{2}[ADP] + 2[ATP] / ([AMP] + [ADP] + [ATP]).$$

NOTE that if all adenosine phosphates are ATP, EC = 1·0 ; if all AMP, E·C = 0 ; & if all ADP or ATP = AMP, EC = 0·5

- **EC vs % maximum reaction rate**
- **The two roles for ATP**

FREE ENERGY AND ATP.

How does the energy in ATP specifically get utilized to power reactions in metabolism?

- The laws of Thermodynamics – First law : In any process, the total energy of the systems and the surroundings remains constant energy is not created nor destroyed, however can be transformed from one form to another.

Second Law. : In any process, the entropy of the system and the surroundings increases, Entropy is often thought of as disorder. or randomness.

THE ULTIMATE DRIVING MACHINE:

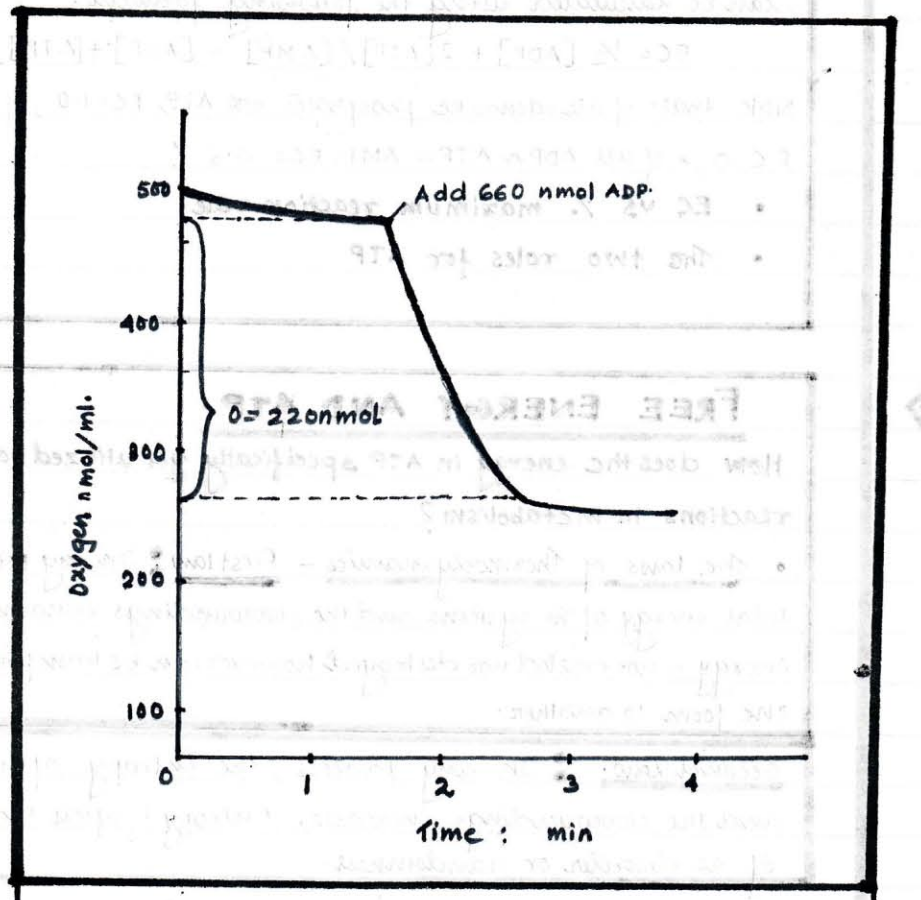
- A New Value for Predicting the Direction of chemical Reactions free energy

$\boxed{DG = DH - TDS}$

G = free energy
H = enthalpy (total energy contained in chemical comp.-ounds)
S = entropy
T = absolute temperature

$A + B \Leftrightarrow C + D$

$DG = DG^{\circ} + RT\ln([C][D]/[A][B])$.



(•) Determination of the P/O Ratio →

Liver mitochondria are incubated in the presence of glutamate. The rate of O_2 uptake from the medium measured by oxygen electrode, is initially low. If ADP is added, respiration speeds up until the ADP is phosphorylated to ATP. The latter can be measured as esterification of Pi. If all of the ADP is esterified, the P/O ratio is 600/220 = 2.7.

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B. The energy carried by ATP is stored in its two terminal phosphate groups.

- ATP is composed of a molecule of adenosine to which three phosphate groups are attached. If one phosphate is removed, ADP (Adenosine diphosphate is produced; if two phosphates are removed, AMP (adenosine monophosphate results).

- At physiologic P^H, ATP is highly negatively charged having a total of three or four negative charges on its phosphates. ATP therefore forms a stable complexes with Mg^{++} and Mn^{++}

- The standard free energy of hydrolysis ΔG°, is approximately -7300 cal/mole for each of the two terminal phosphate groups. Because of this large negative ΔG°, ATP is called a **high energy phosphate compound.**

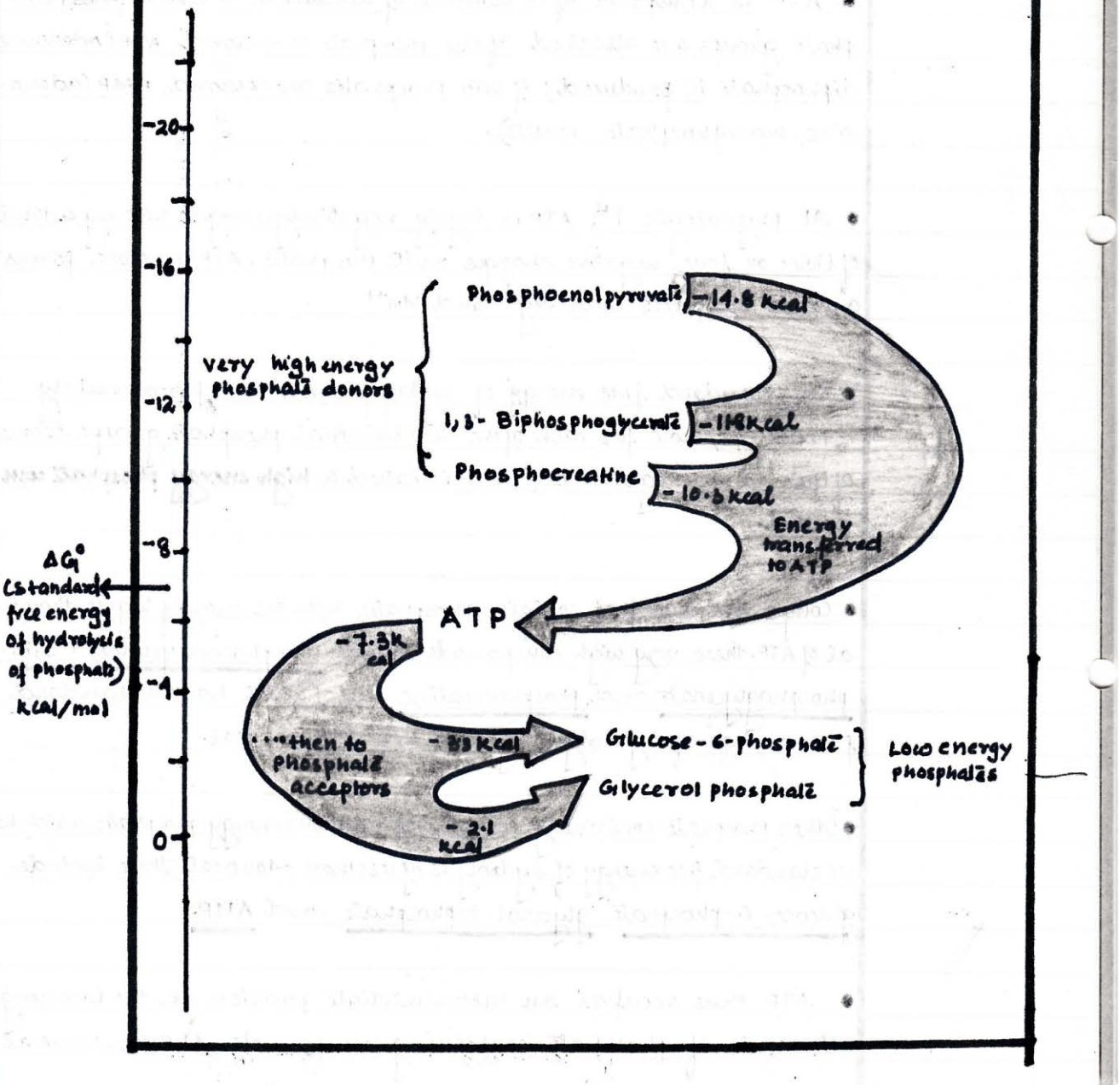
- Compounds exist that contain phosphates with an energy higher than that of ATP. These very high energy compounds include phosphoenolpyruvate, 1-3, bisphosphoglycerate and phosphocreatine, all of which have a standard free energy of hydrolysis greater than -10,000 cal.

- Other phosphate containing compounds have low energy phosphates which have standard free energy of hydrolysis of less than -4000 cal. These include glucose-6-phosphate, glycerol-3-phosphate and AMP.

- ATP thus occupies an intermmediate position on the bioenergetic scale of phosphate containing compounds. ADP can serve as

(Figv) (•)

ATP carries energy between high & low energy compounds.



an acceptor of phosphate groups from cellular phosphates containing higher energy phosphates. ATP can donate these phosphates to compounds in the cell forming phosphates of lower energy (fig V). There are no enzymes in cells that can transfer phosphate groups directly from very high-energy donors to low-energy acceptors without their first being transferred to ATP.

(•) ATP IS THE UNIVERSAL CURRENCY OF FREE ENERGY IN BIOLOGICAL SYSTEMS.

- The central role of ATP in energy exchanges in biological systems was perceived by **Fritz Lipman and by Herman Kalckar in 1941.**
- ATP is a nucleotide consisting of an adenine, a ribose and a triphosphate unit. In considering the role of ATP as an energy carrier, we can focus on its triphosphate moiety. ATP is an rich molecule because its triphosphate unit contains two phosphoanhydride bonds.

$$ATP + H_2O \rightleftharpoons ADP + P_i + H^+ \quad \{\Delta G^{0'} = -7.3 \text{ kcal/mol}\}$$

$$ATP + H_2O \rightleftharpoons AMP + PP_i + H^+ \quad \{\Delta G^{0'} = -7.3 \text{ kcal/mol}\}$$

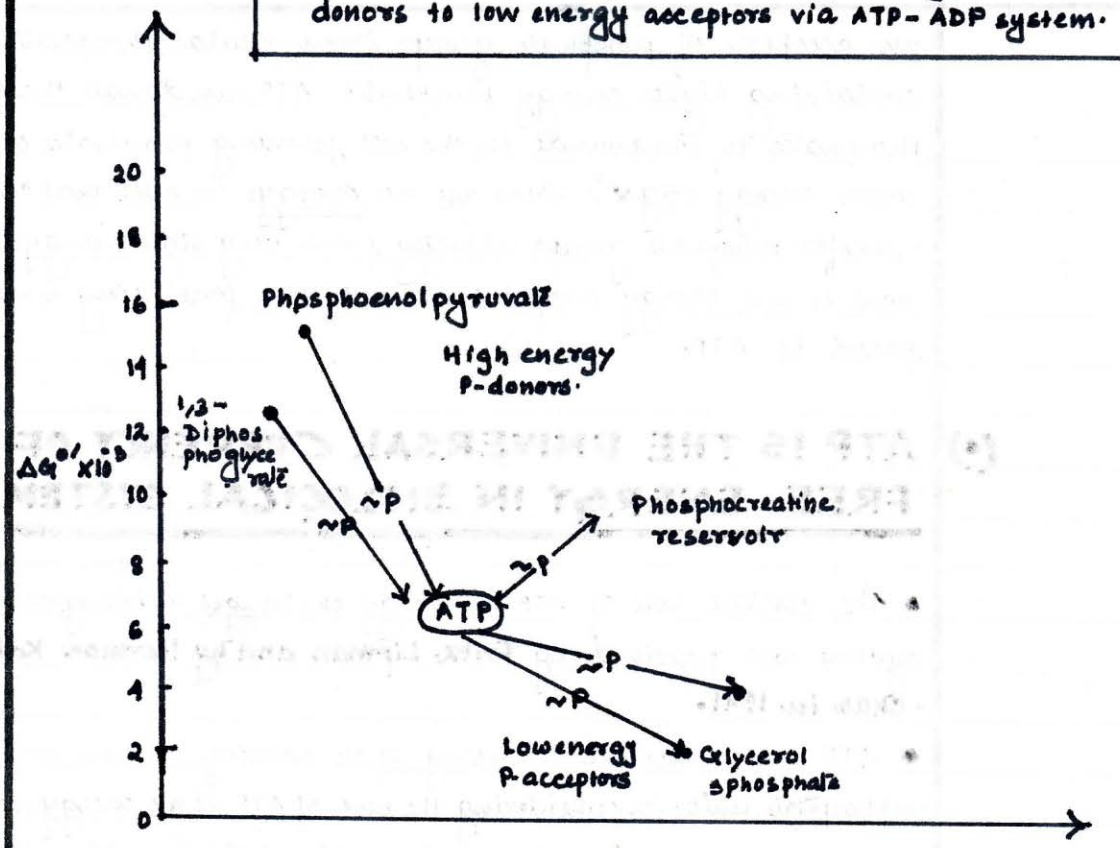
- ATP, AMP and ADP are interconvertible. The enzyme adenylate kinase (myokinase) catalyzes the reaction.

$$ATP + AMP \rightleftharpoons ADP + ADP$$

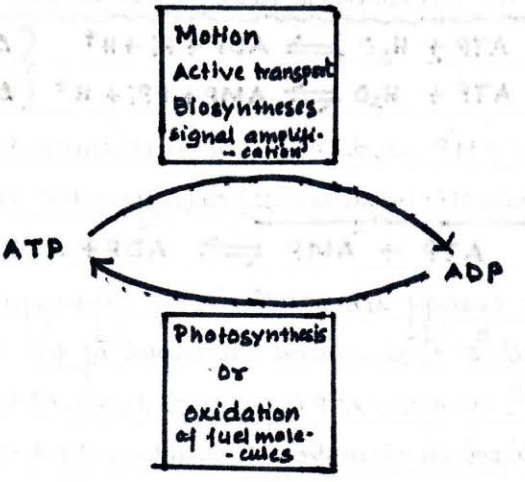
The free energy liberated in the hydrolysis of ATP is harnessed to drive reactions that require an input of free energy, such as muscle contraction. In turn ATP is formed from ADP & P_i when fuel molecules are oxidized in chemotrophs or when light is trapped by phototrophs. **This ATP-ADP cycle is the fundamental mode of energy exchange in biological systems.**

(Fig vi) →

(•) Flow of phosphate groups from high-energy phosphate donors to low energy acceptors via ATP-ADP system.



(Fig vii) →



(•) The ATP-ADP cycle is the fundamental mode of energy exchange in biological systems

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CENTRAL ROLE OF ATP-ADP CYCLE

:ATP IS CONTINUOSLY FORMED AND CONSUMED :-

- ATP serves as the principle immediate donor of free energy in biological systems rather than as a long term storage form of free energy.

- In a typical cell, an ATP molecule is consumed within a minute following its formation. **the turnover of ATP is very high.**

- Motion, active transport, signal amplification and biosyntheses can occur only if ATP is continuously regenerated from ADP. (fig vii)

- Phototrophs harvest the free energy in light to generate ATP, whereas chemotrophs from ATP by the oxidation of fuel molecules.

- In effect an ATP/ADP cycle connects those processes which generate ~ Ⓟ to those processes that utilize ~ Ⓟ

- The processes that feed ~ Ⓟ into this cycle involves -.

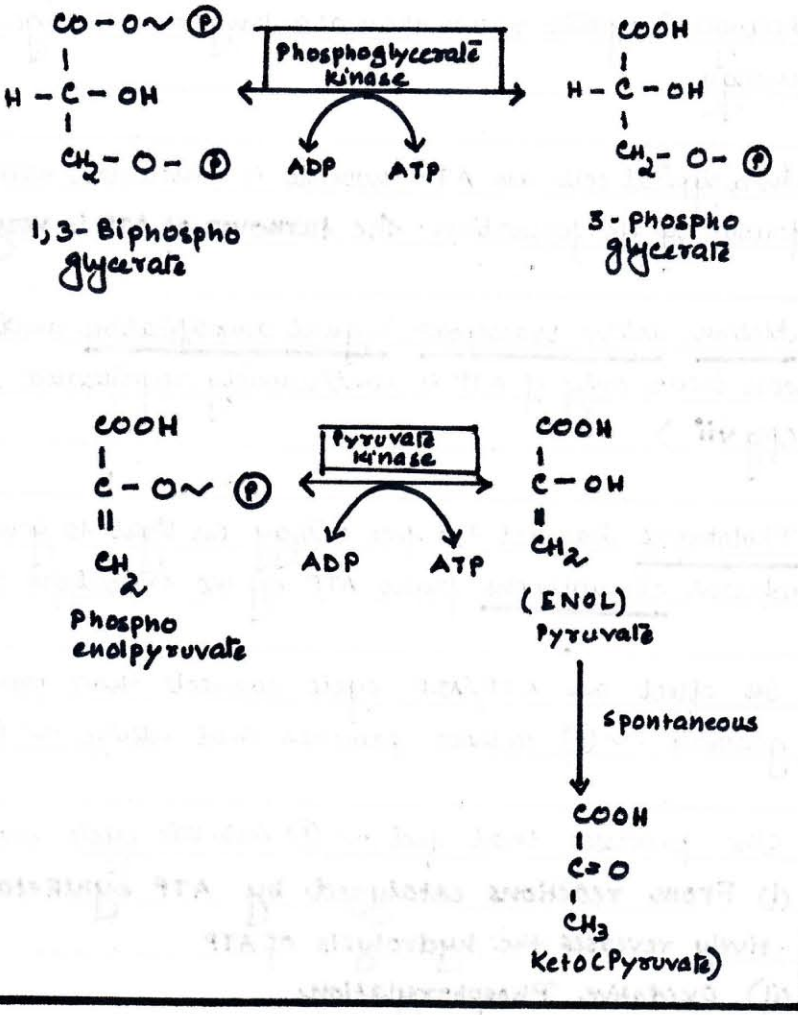
(i) From reactions catalyzed by ATP synthetase which effectively reverses the hydrolysis of ATP

(ii) Oxidative Phosphorylation

(iii) Embden Myerhof Parnas Pathway

(iv) Incorporation of P_i into 3-phosphoglyceraldehyde which after dehydrogenation forms 1,3-biphosphoglycerate.

(Fig viii) (a) Transfer of high energy phosphate from intermediates of EMP to ADP.



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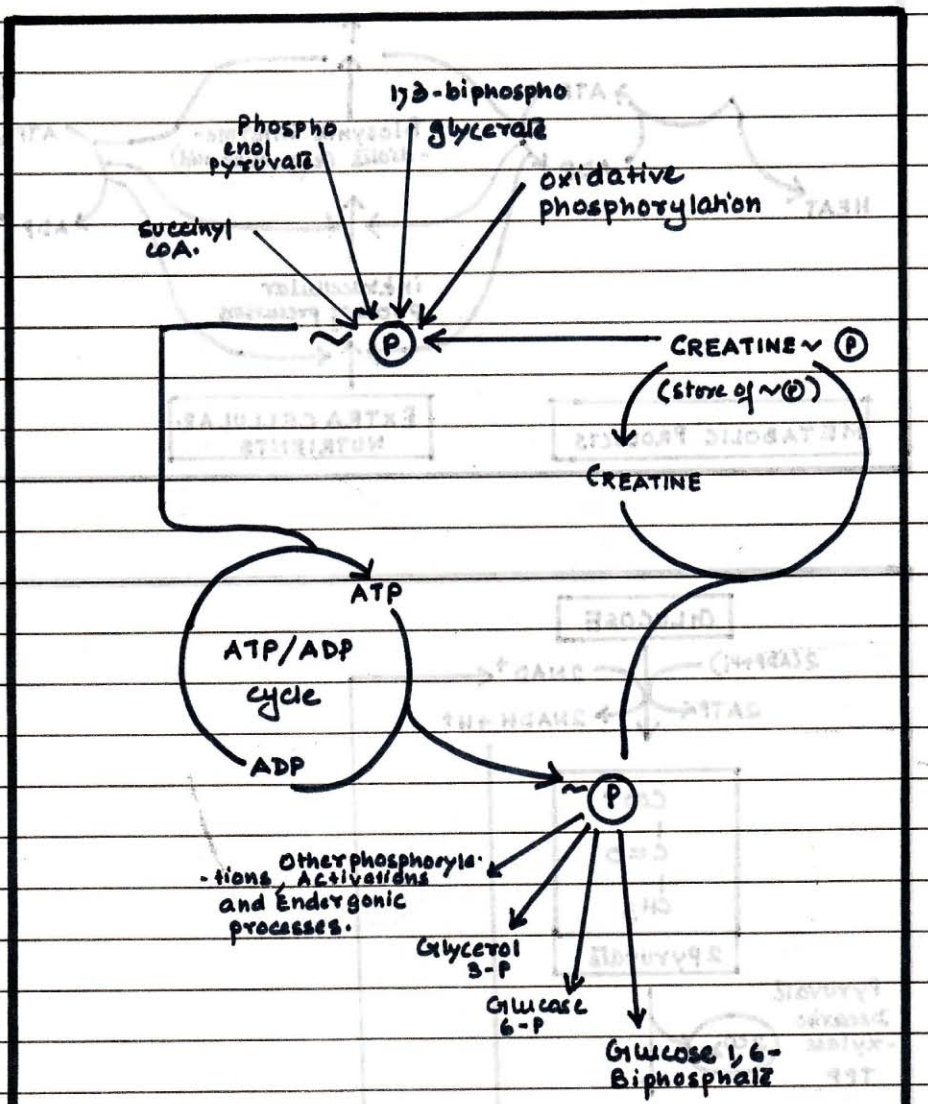
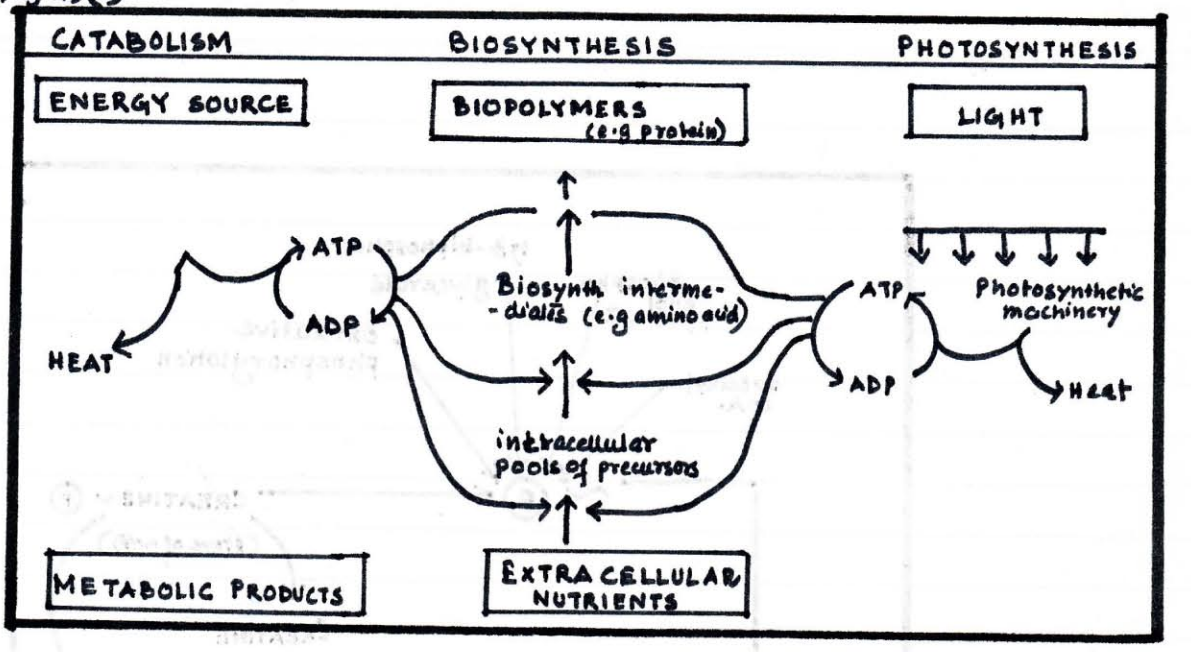
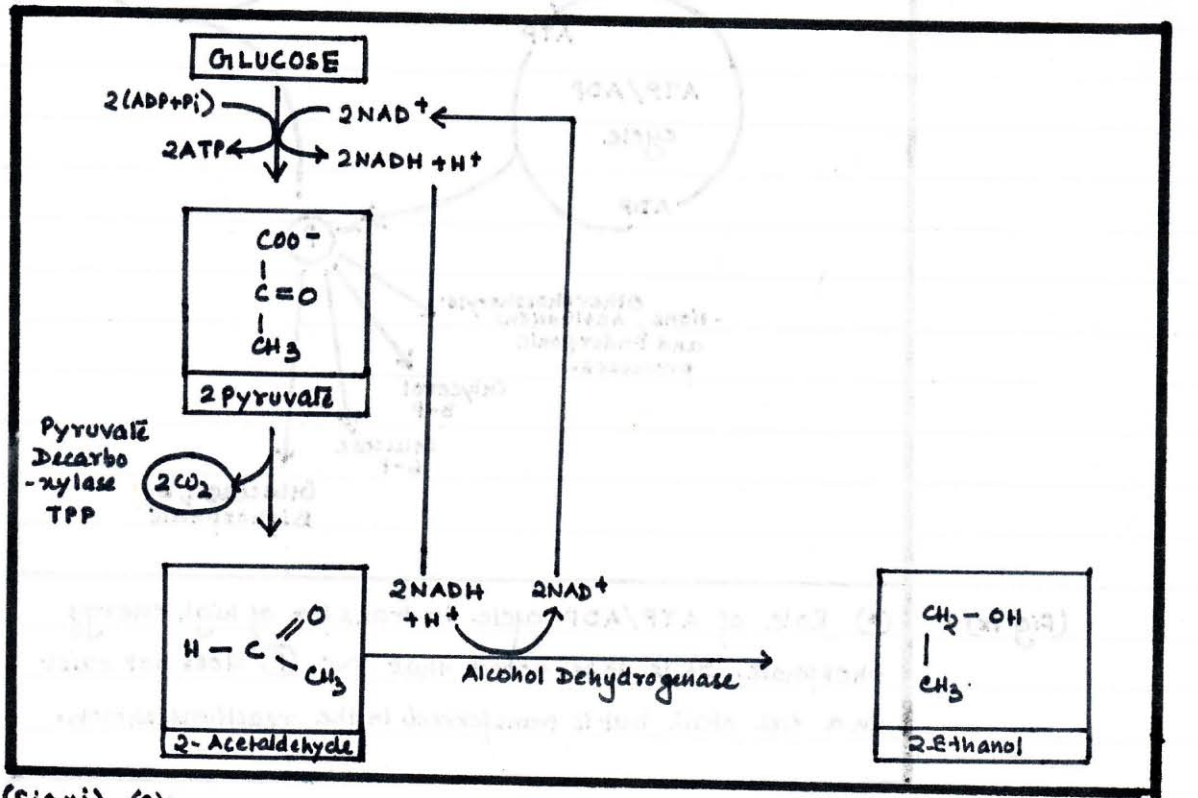


(Fig ix). (•) Role of ATP/ADP cycle in transfer of high energy phosphate. It is to be noted that ~ (P) does not exist in a free state but is transferred in the reactions shown.

(Fig x) (•)





(Fig xi) (•) YIELD OF ATP FROM ALCOHOLIC FERMENTATION.

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Modes Of Energy Yielding Metabolism.

— Generation of ATP is the fundamental mechanics by which some free energy can be trapped.

— In fact most is dissipated in the form of heat. The role of ATP in coupling energy to biosynthesis is summarised in the fig (✱.) aside.

COMPARISON OF FERMENTATⁿ/RESPIRATⁿ/P.Synthesis.

- **Fermentation → Its Nature and Definition.**

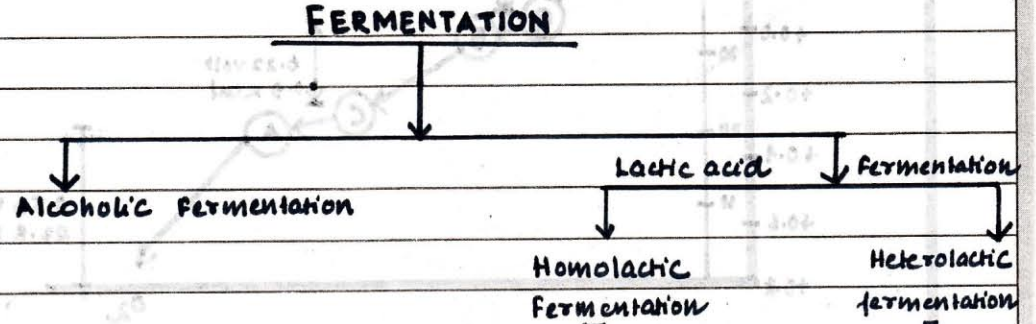
— In the strict sense, the term fermentation refers to those energy yielding pathways in which organic compounds act as both electron donors and electron acceptors

— During fermentation micro-organisms obtain energy from organic compounds without utilizing oxygen.

— The process of fermentation take place in two stages:

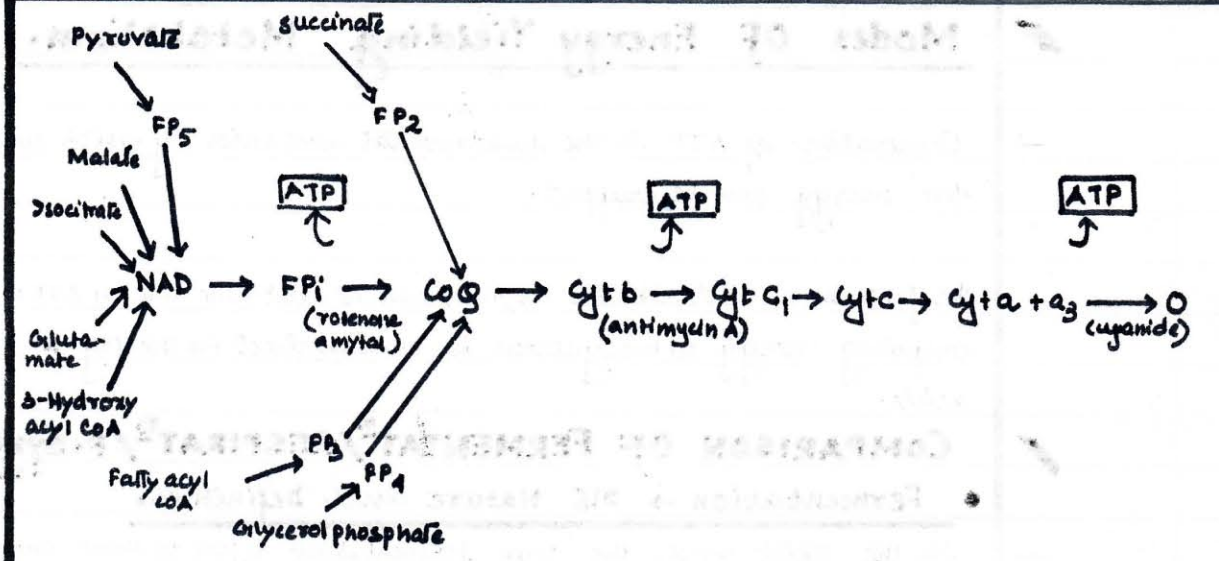
(1) Glucose is broken down to pyruvate with the release of two pairs of hydrogen atoms

(2) Pyruvate or compounds derived from pyruvate are reduced by the hydrogens released in the first stage.



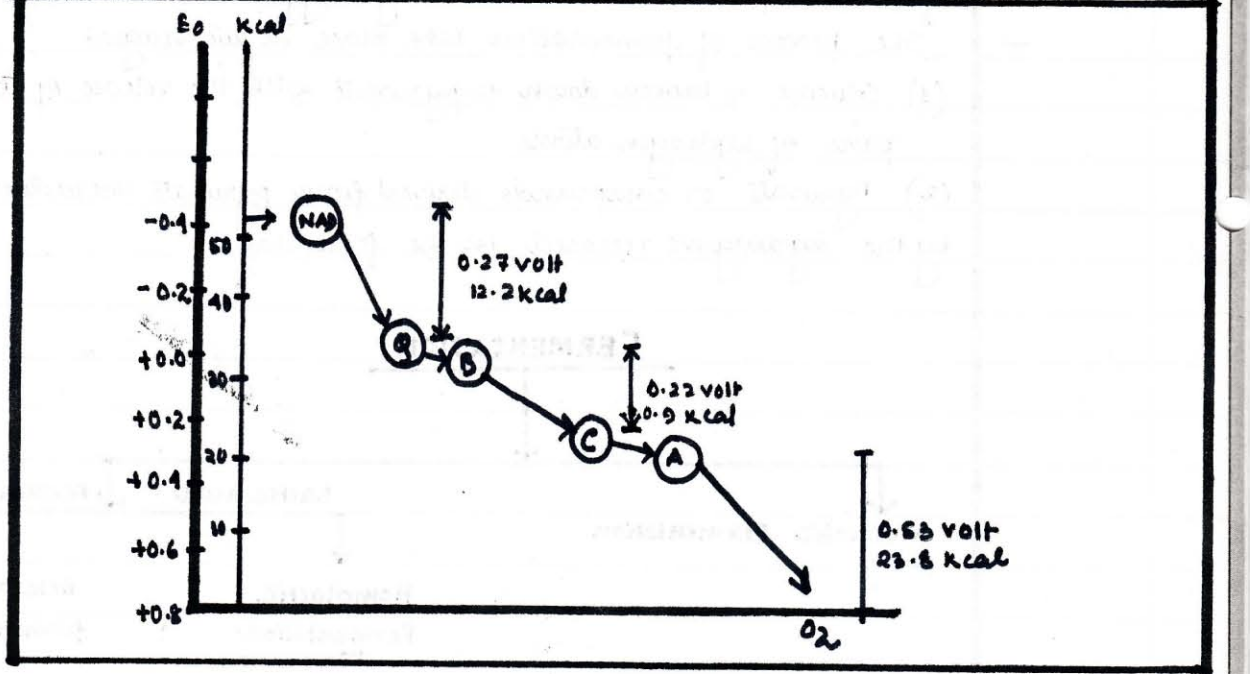
(Fig xii)

(•) The respiratory chain and the pts of entry of electrons from various substrates. Also shown the probable sites of energy conservation leading to ATP formation.



(Fig xiii)

(•) The decline in free energy as electron pairs flow down the respiratory chain to oxygen. Each of the three segments denoted in color yields sufficient energy to generate a molecule of ATP from ADP and phosphate



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RESPIRATION

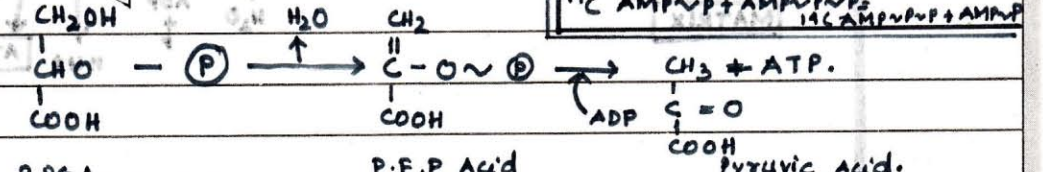
— Respiration is another major energy yeelding reactn

→ Oxidative Phosphorylation

→ Electron transport chain.

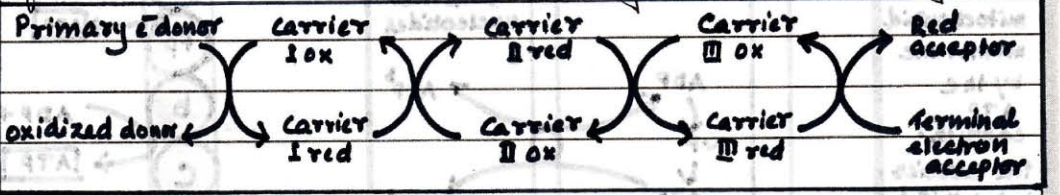
(•) **Synthesis from substrate level Phosphorylation**

— ATP is formed from ADP by transfer of ΔG° PO_4 gr in substrate level phosphorylation



(•) **Synthesis by ETC.**

— ATP is synthesized by transporting electrons through a carrier of molecules with fixed orientation in the cell membrane is a number of microbial metabolic process including respiration & Photosynthesis



— Each number of the chain is capable of being reduced by reacting with the carrier molecule that proceeds it and oxidized by the carrier that follows it.

Partial reactions of oxidative phosphorylation:

The isotopically labelled component is represented. AMP~P~P represents ATP > AMP~P → ADP.

1) ATPase activity.

$ATP + HOH \rightleftharpoons ADP + P_i$

2) ATP Phosphate exchange

$AMP\sim P\sim P + {}^{32}P \rightleftharpoons AMP\sim P_{\sim}{}^{32}P + P_i$

3) Phosphate water oxygen exchange

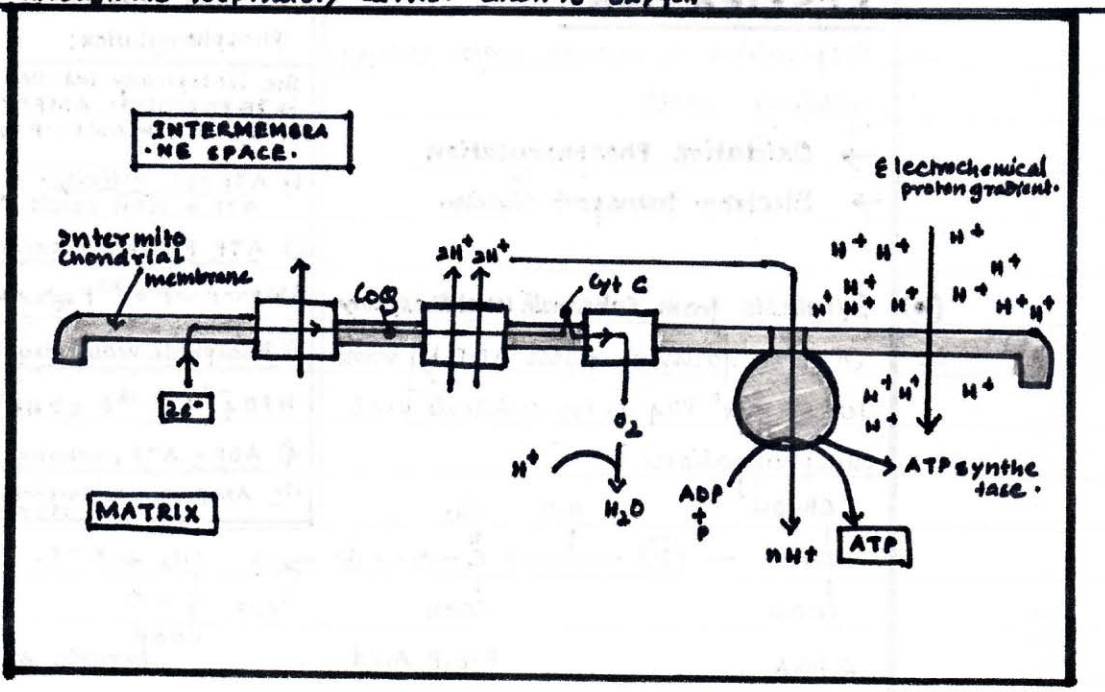
$HPO_4^{-2} + H_2{}^{18}O \rightleftharpoons HP^{18}O_4^{-2} + H_2O$

4) ADP - ATP exchange.

$^{14}C\ AMP\sim P + AMP\sim P\sim P \rightleftharpoons {}^{14}C\ AMP\sim P\sim P + AMP\sim P$

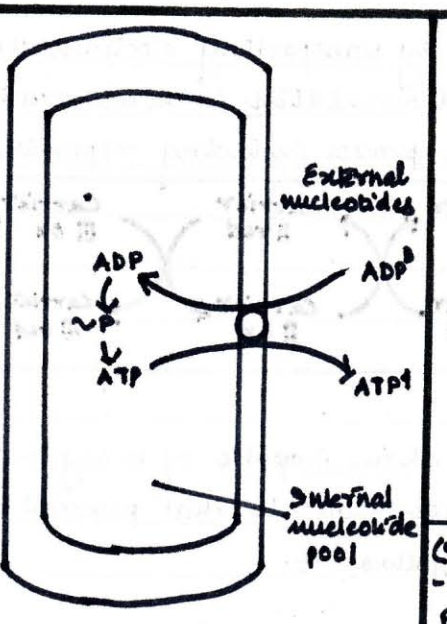
(a) Schematic illustration of the coupled processes of electron transport and oxidative phosphorylation. Using the proton motive force of the electrochemical proton gradient generated by the pumping of protons across the mitochondrial inner membrane. ATP synthetase catalyzes the synthesis of one ATP molecule for each pair of protons pumped out. In this way 3 molecules of ATP are made for the 3 pairs of electrons pumped out as one pair of electrons is transported through the respiratory carrier chain to oxygen

(Fig xiv)

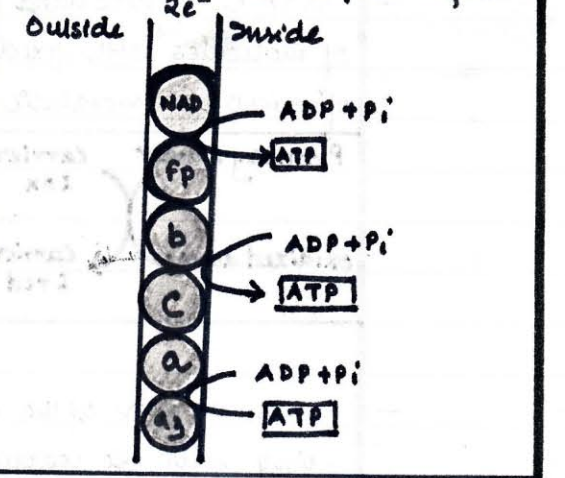


(Fig xv)

(a) Exchange of ADP & ATP across the inner mitochondrial membrane by the ATP carrier. The carrier is inhibited by very low conc^ns of atractyloside which bears some resemblance to the ATP molecule



Oxidative Phosphorylation of ADP



(a) Phosphorylation of ADP and accumulation of cations are alternative processes during electron transport.

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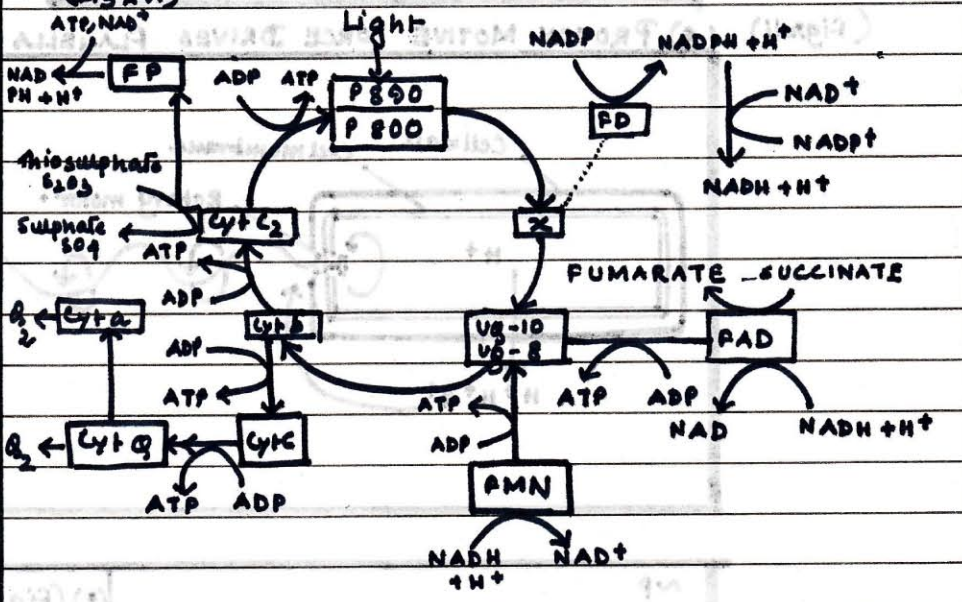
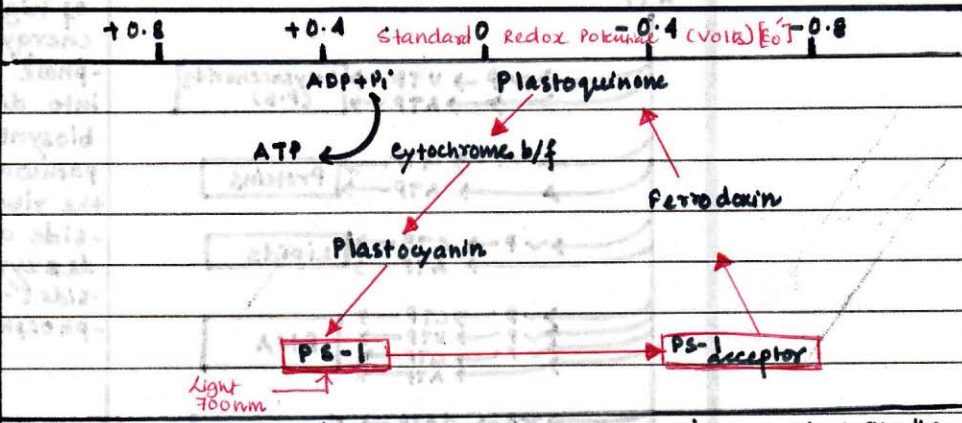
Photosynthesis →

Schematic Representation for electron transport in photosynthetic bacteria under aerobic & anaerobic conditions :-

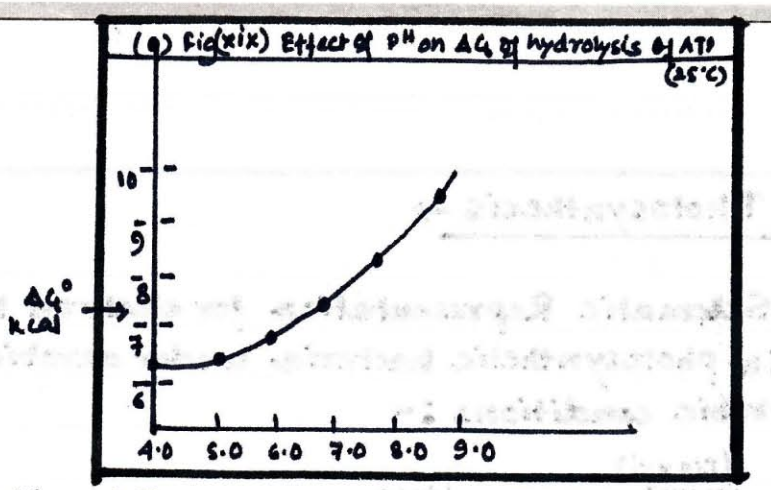
(Fig xvi)



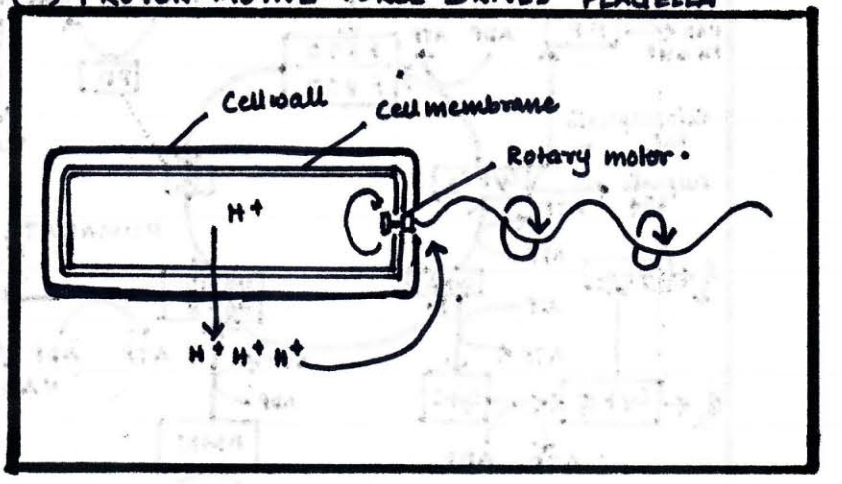


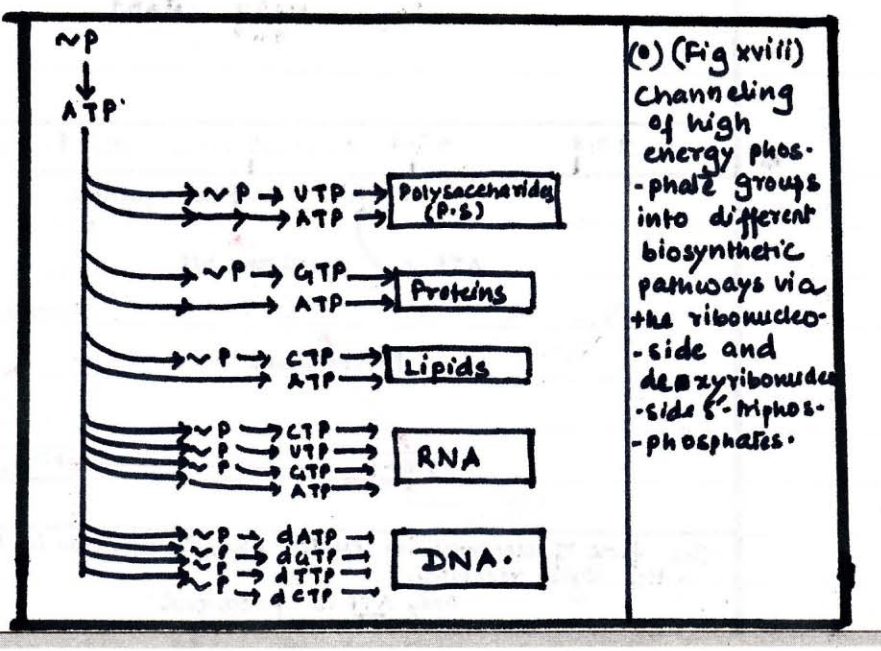
The flow of electrons in cyclic phosphorylation in the photosynthetic light reaction.
Only ATP is produced.

(e) Fig(xix) Effect of pH on ΔG of hydrolysis of ATP (25°C)



(Fig xvii) (e) PROTON MOTIVE FORCE DRIVES FLAGELLA





(e) (Fig xviii) Channeling of high energy phos-phate groups into different biosynthetic pathways via the ribonucleo-side and deoxyribonucle-side 5'-triphos-phosphates.

Expt. No. ..... Page No. .....

ROLE OF ATP IN FLAGELLAR MOVEMENT:-

— Bacterial flagella filaments appear to have no machinery for interconverting chemical and mechanical energy. For example **flagellin**, the flagellar protein molecule, has no enzymatic activity i.e no detectable ATPase activity (such as is present in cilia and flagella of eucaryotic micro-organisms) (Fig xvii) → aside.

- **It is therefore a misconception that in prokaryotic flagellar movement ATP plays a role. Rather here "proton motive force" comes into play**
- **Generally in eukaryotic ciliary & flagellar movement ATP may play a significant role.**

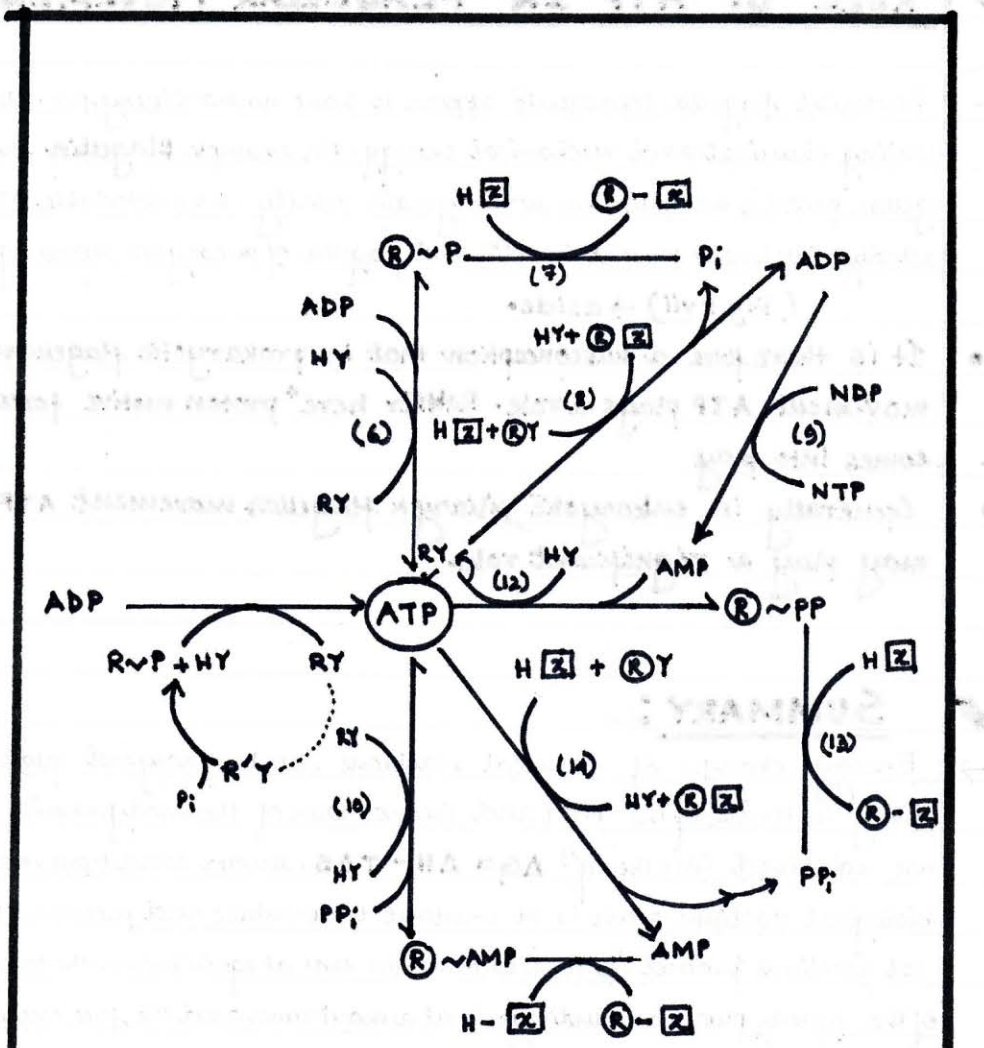
SUMMARY:

→ Energy changes of chemical reactions can be analyzed quantitatively in terms of the First and Second laws of thermodynamics, which are combined into the eqⁿ **$\Delta G = \Delta H - T\Delta S$**. Under conditions in which biological reactions occur i.e at constant temperature and pressure, chemical reactions proceed in such a direction that at equilibrium the entropy's of the system plus surroundings is at a maximum and the free energy G of the system alone is at a minimum. Every chemical reaction has a characteristic standard free energy G_T of the system alone is at a minimum. Standard temperature and pressure with all reactants and products at 1 M concⁿ and $P^H = 7$.

→ ATP is the energy currency of cell.

→ ATP is generated by Respiration, Photosynthesis and Fermentation

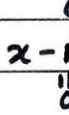
→ ATP is vital for all biological life processes.



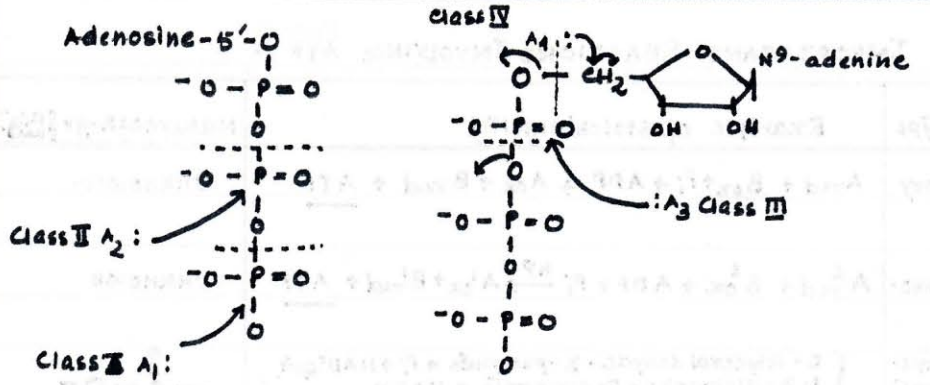
(o) Fig(xx) Schematic Representation of the functions of ATP. Numbers in parentheses refer to reactions in Table given aside (Fig xxi) Bonds designated by ~ are characterized by large negative $\Delta G^{o'}$ of hydrolysis. N refers to a nucleoside.

Expt. No. ..... Page No. .....

(*)(Fig xxi) :- IMPORTATANT REACTIONS INVOLVING ATP :-

Reaction or reaction type	Example of stoichiometry	Nature of R-gr [Fig]
Oxidative phosphorylation.	$A_{red} + B_{ox} + P_i + ADP \rightarrow A_{ox} + B_{red} + \underline{ATP}$	Unknown.
Photosynthetic phosphorylation.	$A^1_{red} + B^1_{ox} + ADP + P_i \xrightarrow{h\nu} A^1_{ox} + B^1_{red} + \underline{ATP}$	Unknown
Triose-Phosphate dehydrogenase plus glycerate kinase	D-Glyceraldehyde-3-phosphate + P_i + NAD^+ ⇌ 1,3-diphospho-D-glycerate + NADH 1,3-Diphospho-D-glycerate + ADP ⇌ 3-phospho-D-glycerate + ATP	
Enolase (phosphoenol pyruvate hydratase)	2-Phospho-D-glycerate ⇌ phosphoenolpyruvate + H_2O Phosphoenol pyruvate + ADP ⇌ pyruvate + ATP	
α-Oxoglutarate dehydrogenase plus succinate: CoA ligase (GDP) plus nucleoside diphosphate kinase or plus succinate: CoA ligase (ADP)	α-oxoglutarate + NAD^+ + CoASH → succinyl-SCoA + NADH. Succinyl-SCoA + GDP + P_i ⇌ succinate + GTP + CoASH GTP + ADP ⇌ GDP + ATP Succinyl-S-CoA + ADP + P_i ⇌ succinate + ATP + CoASH	
Various kinases (ATP: donor phosphotransferases)	ATP + acetate ⇌ acetyl phosphate + ADP (+H_2O)	
	ATP + creatine ⇌ creatine phosphate + ADP (+H_2O)	
Acyl transferases (e.g. phospho-transacetylase)	Acetyl phosphate + HSCoA ⇌ acetyl-SCoA + P_i	
Various synthetases (X:R) ligases	ATP + L-glutamate + L-cysteine ⇌ γ-L-glutamyl-L-cysteine + ADP + P_i	
Nucleoside diphosphate kinases (ATP: nucleoside diphosphate phosphotransferase)	ATP + NDP ⇌ ADP + NTP	P_i.
Various nucleotidyl transferases	ATP + FMN ⇌ FAD + PPi	

(•) Fig(xxii) Donor Functions of ATP

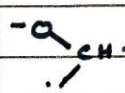
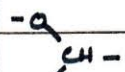
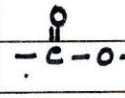


(•) Fig (xxiii)

Class ATP acts as donor of	Formation of	Example.
I. Phosphate (phospho-transferases, 2.7-2.7.4)	A. Anhydride or equivalent ($\Delta G' \geq 0$ kcal/mole)	Acetate + ATP ⇌ acetyl phospha-tase + ADP
	B. Ester, amide or equivalent ($\Delta G' < 0$ kcal/mol)	D-glucose + ATP ⇌ D-glucose-6-P + ADP
II. Pyrophosphate (pyro-phosphotransferases, 2.7.6)	A. Acceptor pyrophosphate	ATP + D-ribose-5-P ⇌ AMP + PR-PP
III. Adenosine-5'-phosphate	A. Enzyme-bound acyl adenylate and transfer to acceptor (6.1.1, 6.2.1)	ATP + RCO_2^- + enz ⇌ [enz. RCO_2^-. AMP] + PPi
		[enz. R-C(=O)-AMP] + CoASH ⇌ R-C(=O)-SCoA + AMP + enz
	B. Dinucleotide coenzymes (2.7.7)	ATP + FMN ⇌ FAD + PPi
	C. Polyadenylate (2.7.7)	(nATP) ⇌ $(AMP)_n$ + n PPi
IV. Adenosine	S-adenosyl methionine (2.4.2.1.a)	ATP + methionine ⇌ S-adenosyl methionine + trimetaphosphate
V. Driving force for reaction	Synthetases / Ligases (class 6)	

Expt. No. ..... Page No. .....

Table (continued.....)

Reaction or reaction type	Example of stoichiometry	Nature of R [gr] (fig xx)
Various ligases (synthetases of groups 6.1 and 6.2, first step)	ATP + enz + RCO_2^- ⇌ [enz·R–C(=O)–O–AMP]	[enz·R–C(=O)–O–]
Various ligases (second step)	[enz·R–C(=O)–O–AMP] + HX ⇌ AMP + R–C(=O)–O–X	[enz·R–C(=O)–O–]
ATP : D-ribose-5-phosphate pyrophospho transferase	ATP + D-ribose-5-phosphate ⇌ AMP + 5phospho-α-D-ribosylpyrophosphate (PRPP)	
Various NMP pyrophosphorylases, pentosyltransferases	PRPP + orotate ⇌ orotidine-5′-phosphate + PP_i	
Various ligases (gr 6.3)	ATP + L-pantoate + β-alanine ⇌ L-pantothenate + AMP + PP_i	
	ATP + xanthosine-5′-phosphate + NH_3 ⇌ GMP + AMP + PP_i	

READY REFERENCE PATTERN

(R.R. PATTERN)

• TRUMP CARD •

• MICROBIOLOGY •

Year - 2003-2004

Name : Hirmee Himadri Dey..

Class : B.Sc. II nd year R.No. 69

Title : Vitamin B_{12} (Cobalamide) production

Introduction :-

- Rickes et al (1948) - isolated Vit B_{12} from liver concentrate.
- Putrification - Smilt & Parker 1948 ; England.
- Flavobacterium solane active in animal protein assay for the treatment of pernicious anemia.
- Recovery of Vit B_{12} from Streptomyces grisens.

Micro organism used :-

Chiefly actinomycetes and bacteria are used

- ACTINOMYCETES :- Nocardia spp ; Streptomyces albidoflavarus - antibioticus - aurofaciens - colobiences - griseus - olivaceus
- BACTERIA :- Aerobacter aroganes ; Bacillus megaterium ; Flavobacterium solane, Pseudomonas spp.. etc..

Uses :-

- Human and animal nutrition (medicine and microbiology)
- When introduced in stock feed of 10-15 mg / ton it increases the vegitable protein utilization of poultry, swine etc..
- It increases body weight considerably.
- Stimulate appetite & growth rate in children.
- Effective in treatment of pernicious anemia.

Other method :-

Mycellium separated from whole beer suspended in water.

↓

Reducing the pH suspension to 5 (with H_2SO_4)

↓

Heating it to boiling.

↓

Centrifuging / Filtering

↓

Evaporating the liquor to stir up under vaccum.

↓

Drying

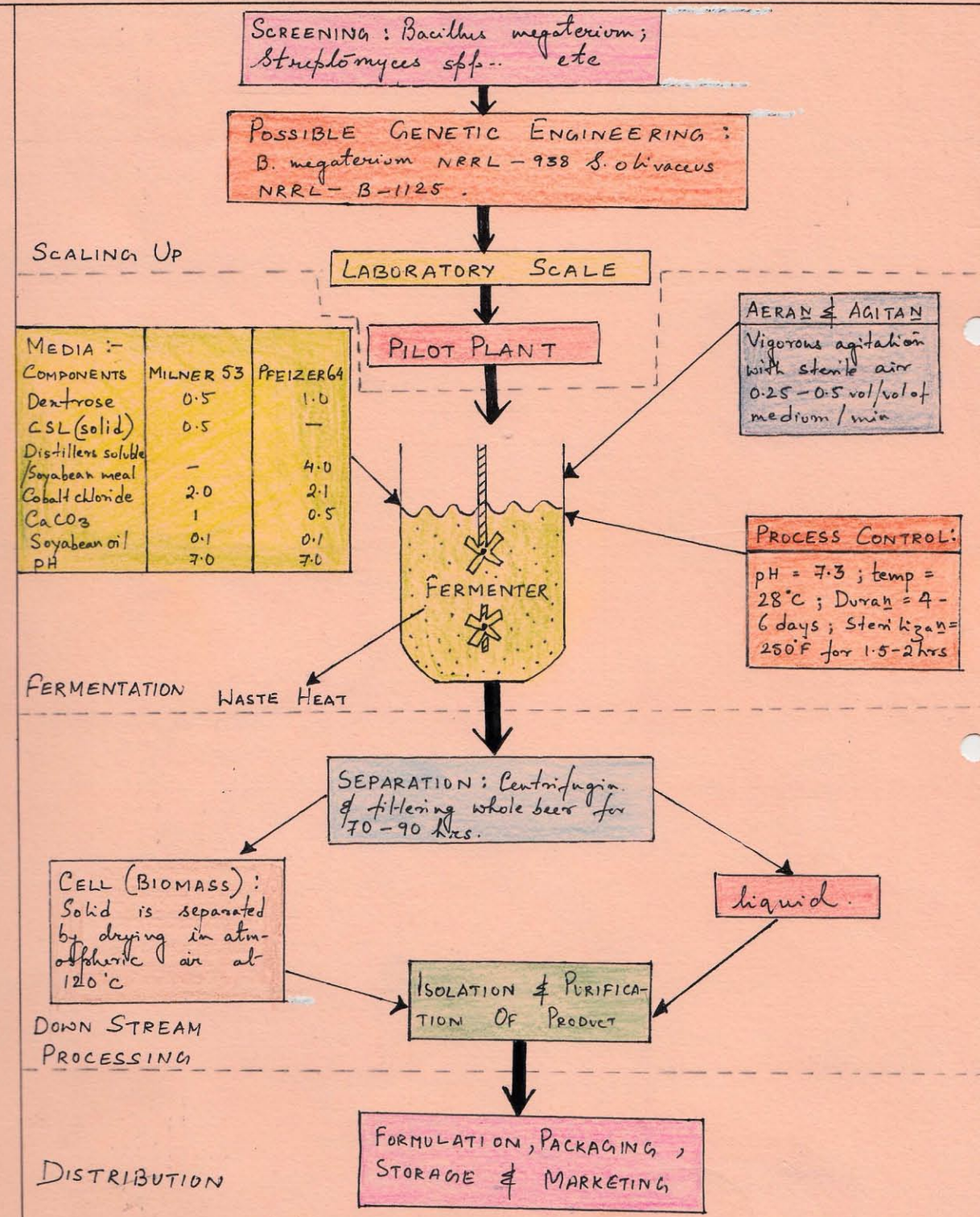
↓

Vit B_{12} / 100 mg / pound

Ready Reference Pattern : Trump Card

Title : Vitamin B_{12} (Cobalamide) prodn:

No. of Books/Journals/ Websites Reffered : 6.



–: Fermentation Procedure :–

READY REFERENCE PATTERN

(R.R. PATTERN)

• TRUMP CARD •

• MICROBIOLOGY •

Year - 2003 - 2004

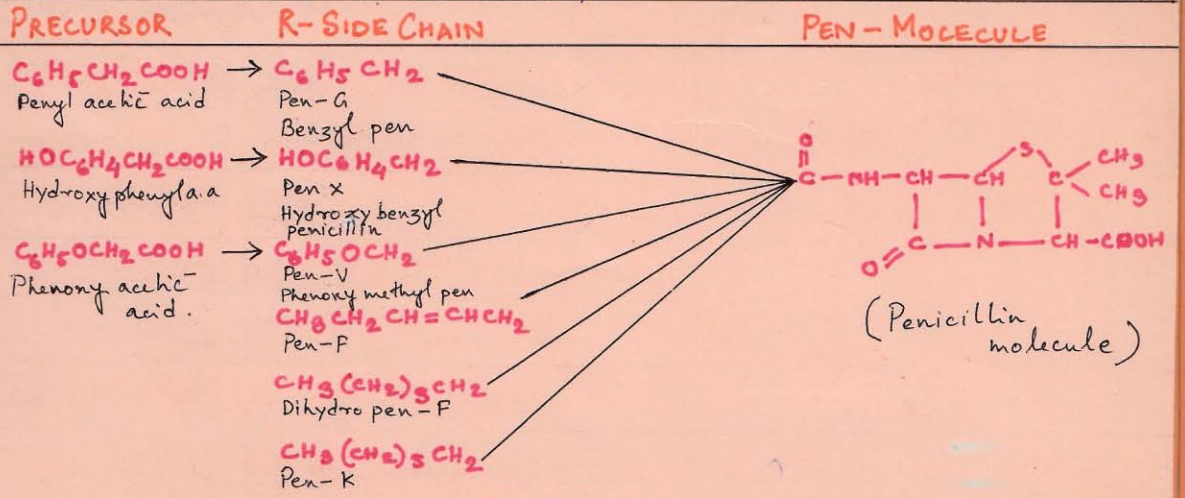
Name : Hoimee Dey

Class : B.Sc. 1nd yr. R.No. 69

Title : PENICILLIN PRODUCTION

1. **INTRODUCTION :-** Penicillin is the first antibiotic produced on large scale. It is active against Gr +ve bact.. but rarely against Gr-ve ones. It hampers cell wall synthesis and is almost non toxic to mammals except for the allergic reactions.

2. **HISTORY :-** It was first observed by Sir Alexander Fleming (in 1929) accidentally while studying air microflora. He observed Pen-F produced by Penicillium notatum. Later Chain et al (in 1940) and Abraham et al (in 1941) published their observations.



3. **PENICILLIN MOLECULE & PRECURSORS (STRUCTURES) :-**

4. **STRAINS USED :-** P. notatum ; P. crysogenum (from mouldy fruits) → mutagenic agents (X rays, UV-rays, MBA etc) → 8-176. Recently still high yielding strains have been discovered.

Ready Reference Pattern : Trump Card

Title : PENICILLIN PRODUCTION.

No. of Books/Journals/ Websites Reffered :

5. FEMENTATION PROCEDURE:

5.1 MEDIA

SPORULATION MEDIA :-

KOFFLER'S SPORULAN MED..		FORSTERS et al's MED		JACKSONS MEDIA	
Peptone	- 5 gm	Sucrose	- 20 gm	Corn sleep liquor	- 3.5 gm
Sugar beet molasses	- 5 gm	$NaNO_3$	- 0.6 gm	Lactose	- 3.5 gm
NaCl	- 4 gm	KH_2PO_4	- 1.5 gm	Glucose	- 1.0 gm
KH_2PO_4	- 0.1 gm	$MgSO_4 \cdot 7H_2O$	- 0.5 gm	$CaCO_3$	- 1.0 gm
$MgSO_4 \cdot 7H_2O$	- 0.05 gm	$CaCl_2$	- 0.25 gm	KH_2PO_4	- 0.4 gm
Agar-agar	- 15 gm	D/W	- 1lt	Edible oil	- 0.25 gm
D/W	- 1lt			D/W	- 0.005 gm
	(1946)		(1946)	Precursors	- 100 ml
					(1958)

INOCULATION MEDIA :-

The oldest one is Czapek & Dox Media. Other known media are Calum Hockenhull (1956), Sylvester & Coghill's media (1954) etc...

5.2 PREPN & INOCUN OF INOCULUM

Suspend spores (from heavily sporulated slants) in water.

Add spores in nutrient soln.

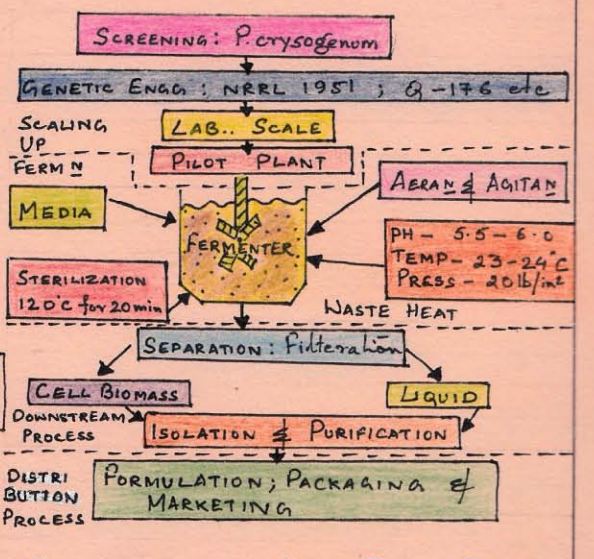
Incubate for 5-7 days at 24°C for sporn

Inoculate directly in tank

Incubate for 24 hrs - 48 hrs in tank with aeration & agitation for heavy growth

Test any contamination

Do not add precursors.



CHARACTERISTIC CHANGES IN 3 PHASE OF PEN.. PRODN:

CONDITIONS	PHASE I (Growth)	PHASE II (Maturation)	PHASE III (Decline)
Pen production	slight	maximal	nil - distruction
pH value	sharp rise	falation / slight fall	raise
Mycelium	rapid growth / High N content	slow growth / Low N content	dec.. in dry wt & N content
Lactose	slow use	rapid use	exhausted
Lactic acid	exhausted rapidly	—	—
Ammonia	released into medium	utilized	released into medium
Nitrate	used slowly	used slowly	used slowly.
Non-ammonia N	used exhaustively	conc. stable	conc.. increased.
Inorganic P	used at max.. rel (slowly)	used slowly.	no use / liberation
O_2 (N)	maximum	decrease	minimum;

READY REFERENCE PATTERN

(R.R. PATTERN)

• TRUMP CARD •

• MICROBIOLOGY •

Year - 2003 - 2004

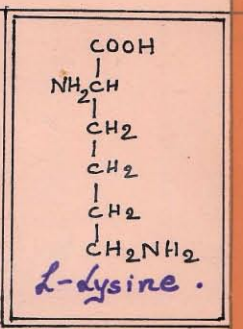
Name : Hoimee Dey...

Class : B.Sc. II nd yr R.No. 69

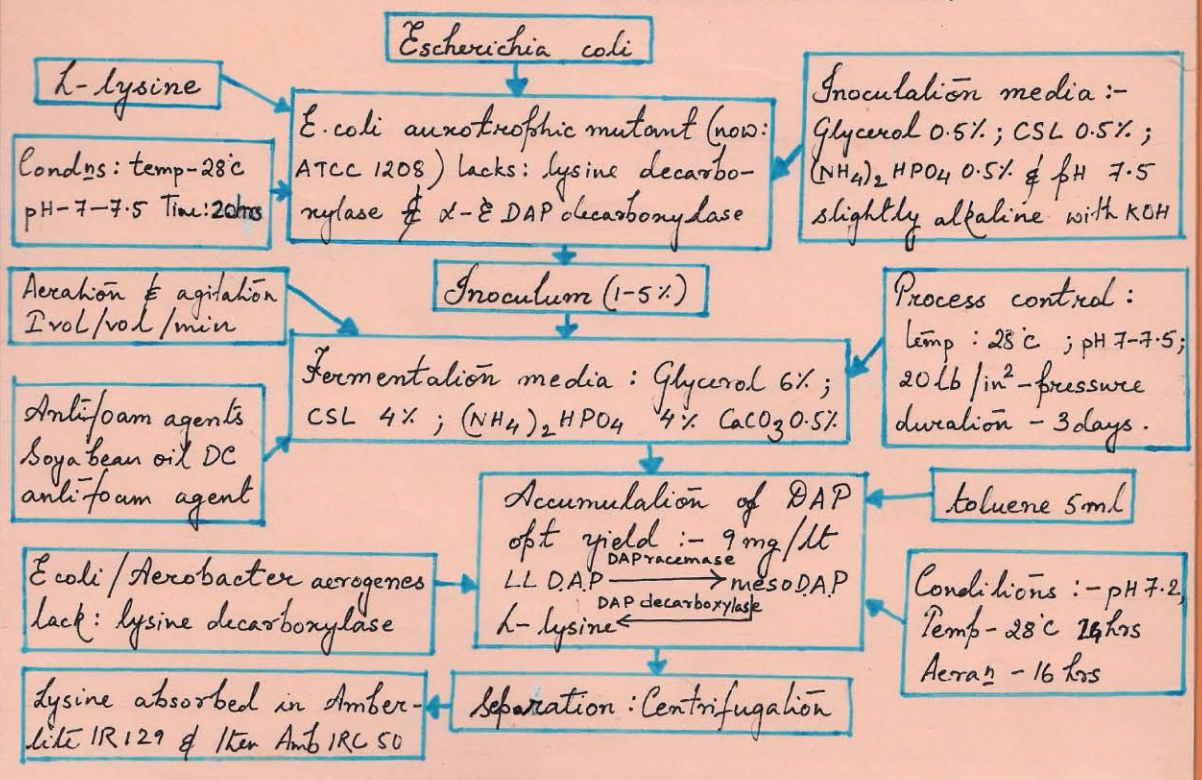
Title : -: L-Lysine Production :-

Introduction & History :-

- EXTRACELLULAR LYSINE : Richard & Haskins (1957) - screened 560 fungi : Medium - Glucose, Urea & Mineral salts.
- Dulany (1957) - by shaken flask techique obtained 400 mg/ml : Mo's : Gliocladium spp & Ustilago maydis.
- First amino acid on a commercial scale by fermn Casida (1956) : Escherichia coli auxotrofic mutant (now :- ATCC 12408) : Indirect method : DAP → L-lysine.
- OTHER D.A.P producing strains
  - Corynebacterium diphtheria ; Mycobacterium tuberculosis (Dr Work 1951).
  - E. coli auxotrofic mutant (Davis 1952).
- Method : Submerged culture techique



-: Indirect or Dual fermentation :-



Ready Reference Pattern : Trump Card

Title : L- Lysine Production

No. of Books/Journals/ Websites Reffered : 4.

The Direct Method (Kinoshita et al 1958)

Micrococcus glutamicus

↓

Auxotroph capable of producing d- Lysine by dual fermⁿ

Duration 4 days

Basal medium:-
Glucose 7·5% $(NH_4)_2SO_4$ 1·5%
K_2HPO_4 0·05% KH_2PO_4 0·05%
$MgSO_4 \cdot 7H_2O$ 0·025% $CaCO_3$ 1%

Cultural med:-
Glucose 10%, $(NH_4)_2SO_4$ 2%,
$CaCO_3$ 2%, L-theronine 400mg/lt
Biotin 7·5 µg K_2HPO_4 0·1%,
$MgSO_4 \cdot 7H_2O$ 0.08% L Meth 300mg

Fermentation : Black strap molasses + Hydrolysed soyabean.

Condⁿs:-
pH = 7 (NH_3)
temp 28°C
Duran - 60hrs

↓

Acidity the classified broth with Hydrochloric acid.

↓

Absorption of lysine in an cation exchange column in ammonium form

↓

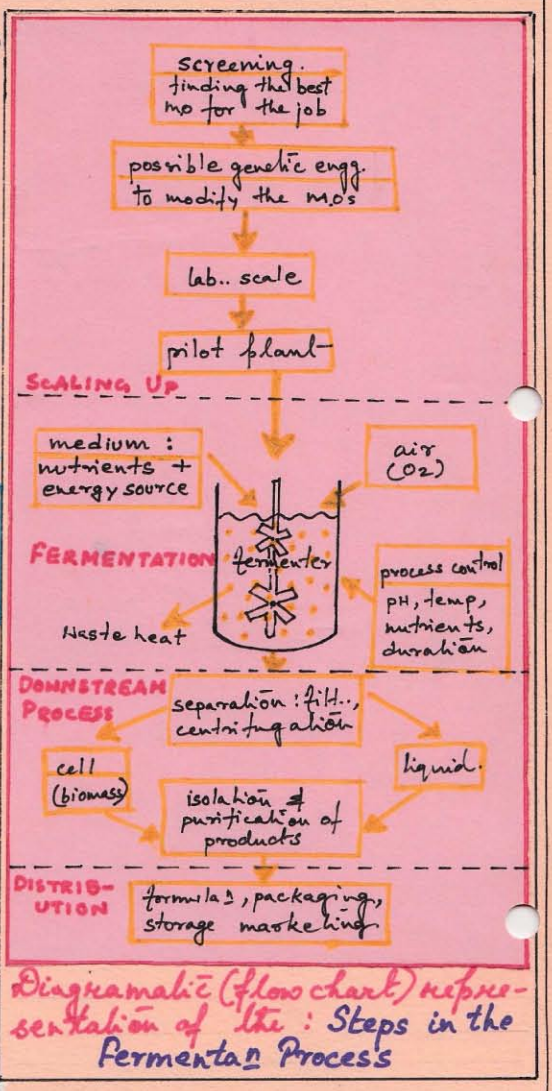
Reacidification with HCl

↓

Crystallisation of L-lysine hydrochloride

↓

Marketing
yield : always >20 gm/lt (min) 40–45 gm/lt (starting with molass conc.. 200gm/lt 160g/lt of sugar.



Diagramatic (flow chart) representation of the : Steps in the Fermentation Process

Uses of L- Lysine :

a) Food supplement

b) Growth factor in number of tonics which prevent protein deficiency diseases (Kwashiorkor)

Important features :-

- Erythromycin encourages the prototrophs
- Biotin added must be in excess.
- Pen.. addition initiates glutamate production in the homoserene less strain