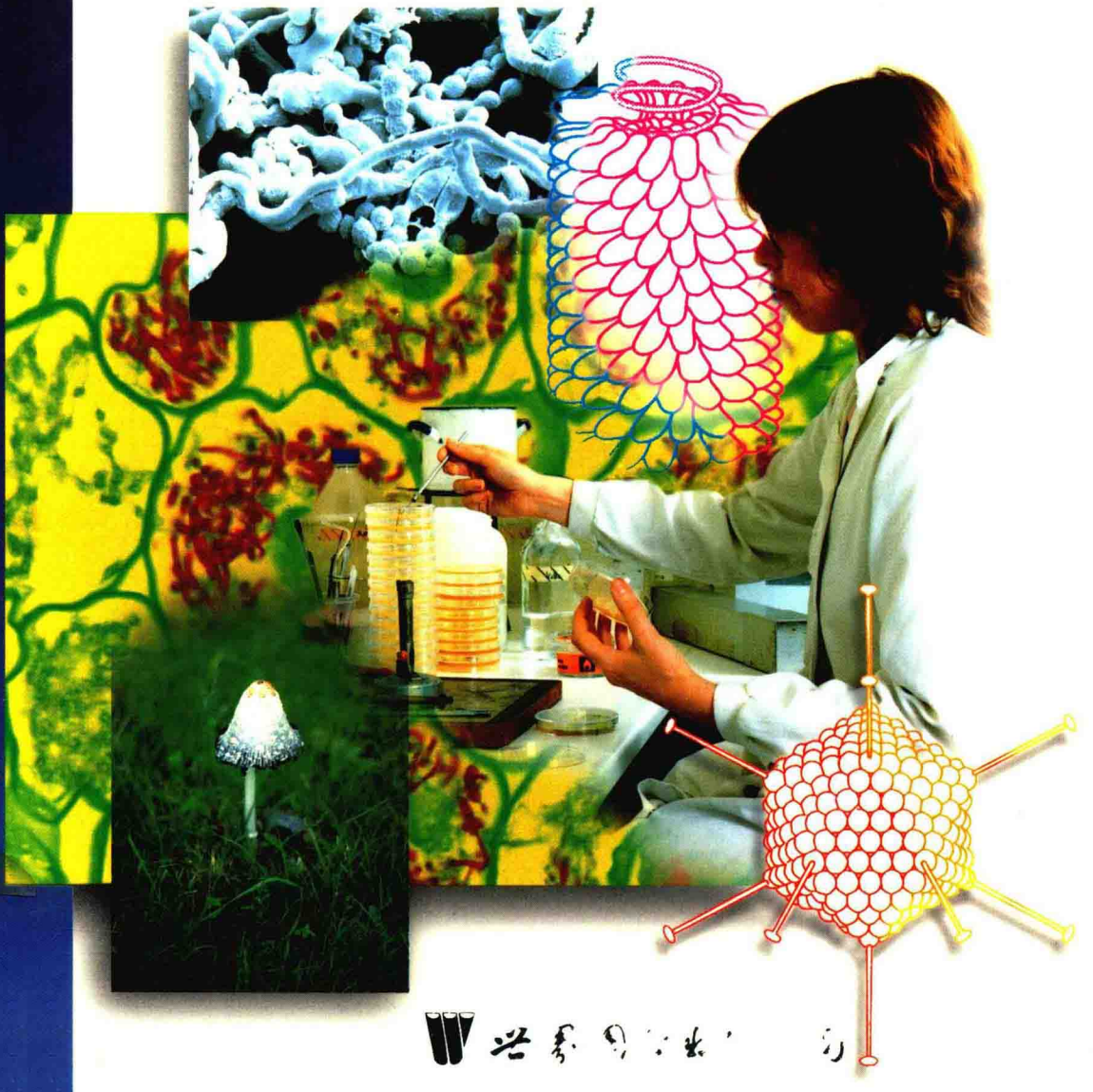
Instant Notes in

MICROBIOLOGY



J. Nicklin, K. Graeme-Cook,
T. Paget & R. Killington

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J. Nicklin
Department of Biology,
Birkbeck College, London, UK

K. Graeme-Cook
Department of Biology,
Birkbeck College, London, UK

T. Paget
Department of Biological Sciences,
University of Hull, Hull, UK

&

R.A. Killington
Department of Microbiology,
University of Leeds, Leeds, UK

BIOS SCIENTIFIC PUBLISHERS

Springer

[a]J. Nicklin, [a]K. Graeme-Cook, [b]T. Paget and [c]R.A. Killington
[a]*Department of Biology, Birkbeck College, London, UK,*
[b]*Department of Biological Sciences, University of Hull, UK and*
[c]*Department of Microbiology, University of Leeds, Leeds, UK*

First published 1999

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A CIP catalogue record for this book is available from the British Library.

ISBN 981-3083-73-5 Springer-Verlag Singapore Pte Ltd

Production Editor: Fran Kingston.
Typeset and illustrated by The Florence Group, Stoodleigh, UK.
Printed by Biddles Ltd, Guildford, UK.

Instant Notes in MICROBIOLOGY

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Series editor
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School of Biochemistry and Molecular Biology, University of Leeds, Leeds, UK

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ABBREVIATIONS

A	adenine
AcCoA	acetyl coenzyme A
ACP	acyl carrier protein
ADP	adenosine 5′-diphosphate
Ala	alanine
AMP	adenosine 5′-monophosphate
A-site	amino-acyl site (ribosome)
ATP	adenosine 5′-triphosphate
ATPase	ATP synthase
BHK	baby hamster kidney
bp	base pair
C	cytosine
C-phase	chromosome replication phase (bacterial cell cycle)
CAP	catabolite activator protein
CAT	chloramphenicol acetyl transferase
CFU	colony-forming unit
CMV	cytomegalovirus
CNS	central nervous system
CPE	cytopathic effect
CRP	cAMP receptor protein
CTL	cytotoxic T lymphocyte
Da	Dalton
D-Ala	D-alanine
DAP	*meso*-diaminopimelic acid
D-Glu	D-glutamic acid
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
DOM	dissolved organic matter
D-phase	division phase (bacterial cell cycle)
ds	double-stranded
EF	elongation factor
EM	electron microscopy
ER	endoplasmic reticulum
FAD	flavin adenine dinucleotide (oxidized)
$FADH_2$	flavin adenine dinucleotide (reduced)
G	guanine
G-phase	gap phase (bacterial cell cycle)
GTP	guanosine 5′-triphosphate
HA	hemagglutination
Hfr	high frequency recombination
HSV	herpes simplex virus
I	inosine
ICNV	International Committee on Nomenclature of Viruses
Ig	immunoglobulin
IHF	integration host factor
Inc group	incompatible group (of plasmids)
IS	insertion sequence
Kb	kilobase
KDO	2-keto-2-deoxyoctonate
KDPE	2-keto-2-deoxy-6-phosphogluconate
Lac	lactose
LBP	luciferin-binding protein
LPS	lipopolysaccharide
MAC	membrane-attack complex
MCP	methyl-accepting chemotaxis protein
MEM	minimal essential medium
MHC	major histocompatibility complex
m.o.i.	multiplicity of infection
mRNA	messenger ribonucleic acid
MTOC	microtubule organizing centre
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
$NADH + H^+$	nicotinamide adenine dinucleotide (reduced form)
NADP	nicotinamide adenine dinucleotide phosphate (oxidized form)
$NADPH + H^+$	nicotinamide adenine dinucleotide phosphate (reduced form)
NAG	*N*-acetylglucosamine
NAM	*N*-acetylmuramic acid
NB	nutrient broth
NTP	ribonucleoside triphosphate
O	operator
OD	optical density
omp	outer membrane protein
P	promoter
PCR	polymerase chain reaction
pfu	plaque-forming unit
PHB	poly-β-hydroxybutyrate

Phe	phenylalanine
P_i	inorganic phosphate
PMF	proton motive force
PMN	polymorphonucleocyte
PP_i	inorganic pyrophosphate
PS	photosystem
PSI and II	photosystems I and II
P-site	peptidyl site (ribosome)
R	resistance (plasmid)
RBC	red blood cell
redox	reduction–oxidation
RER	rough endoplasmic reticulum
rubisco	ribulose bisphosphate carboxylase
RNA	ribonucleic acid
rRNA	ribosomal RNA
S	Svedberg coefficient
snRNA	small nuclear ribonucleic acid
SPB	spindle pole bodies
ss	single-stranded
T	thymine
TCA	tricarboxylic acid
TCID	tissue culture infective dose
tRNA	transfer RNA
Trp	tryptophan
TSB	tryptone soya broth
U	uracil
U_L, U_S	unique long, unique short
UDP	uridine diphosphate
UDPG	uridine diphosphate glucose
UV	ultraviolet light

PREFACE

Microbiological matters have always been in the public eye, but recent occurrences of food poisoning, antibiotic-resistant bacteria, HIV and BSE have heightened awareness of the existence of microbes and emphasized the need for continuing research into microbiology. We need to understand and control the activities of microbes when they are detrimental to mankind, and to enhance and manage their effects when they are beneficial to us.

Very little microbiology is taught in schools within the A-level syllabus, and foundation text books in the subject must deal effectively with the basics of the topic, but must also provide sufficient information to bring the reader's knowledge up to university first- or second-year standards. Many of the available texts are very large and expensive and dwell heavily on the medical, symptom-based aspects of microbiology. This textbook aims to provide the reader with basic information about microbes without this bias.

We have considerable experience in teaching microbiology, especially to mature, part-time undergraduate and postgraduate students at Birkbeck College, University of London. These students, who attend lectures in the evening, have busy life-styles and many commitments. They therefore require information to be presented in a concise, understandable format without excessive frills or diversions. We have written this book using our understanding of their problems.

Instant Notes in Microbiology has been written in a way that gives students easy access to the important key features of microbes. The book has been divided into eleven main sections which cover all areas of microbiology. Each topic starts with a key notes section, which is a revision check list of the topic, and then expands on the subject. Diagrams are kept to the simple outline drawings that a student might produce under examination conditions. Further reading lists are provided for each topic.

In Section A you are introduced to the diverse range of microbes and the roles they can play in the environment. Section B covers the biochemistry of metabolism in microbes and Section C describes salient features of DNA replication, transcription and translation in both prokaryotes and eukaryotes. In section D, bacterial taxonomy, structure, function and growth are discussed and topics on handling bacteria in the laboratory are included. The bacterial genetics section, Section E, is devoted to the means by which bacteria can alter their genetic make-up, including mutagenesis, DNA repair, conjugation, transduction and transformation. Section F, the last on bacteria, discusses their relationship with their environment. This section is heavily slanted to bacterial interactions with a human host as this is still the region of microbiology which is studied the most, and is probably of the greatest interest to the average reader.

Section G introduces the reader to the basic structure of eukaryotic cells, and the taxonomic divisions within the eukaryotic protists. Nuclear (mitotic and meiotic) division and cell division are also covered in this section. Subsequent sections deal with the different taxonomic groups in more detail. Section H describes the fungi, their structure, biology and impact on the environment. Within this section other related phyla are considered, organisms which by parallel evolution have come to share many characteristics of fungi. The

important features of the algae are covered in Section I, and in Section J the biology of the protozoa and their disease-causing capacity is detailed. The final subject of virology is dealt with in Section K.

This book has been designed to allow students instant access to subjects that are written as free-standing topics. Cross-referencing to other topics allows the reader to follow-up different lines of interest, and the reading list provided for each section should provide further sources of information. Due to the many different aspects of microbiology, there are, inevitably, a few omissions from this book, notably the use of microbes in molecular biology. Other books in the *Instant Notes* series, including *Instant Notes in Molecular Biology* and *Instant Notes in Biochemistry*, will help to fill these gaps. However, for all topics related to general microbiology we hope that you will find this book a useful revision aid and a stimulus for further study.

Kate Graeme-Cook and Jane Nicklin

Acknowledgments

We would like to thank our families, colleagues (especially Martin Ingrouille), neighbors and friends for their support and help while we were writing this book. Thanks are also due to the staff at BIOS for their encouragement and assistance during this time. Finally, we would like to thank David Hames for asking us to write the book and whose constructive criticisms greatly improved its general style.

We would like to dedicate this book to our parents.

CONTENTS

A1 THE MICROBIAL WORLD

Key Notes

What is a microbe?

The word microbe (microorganism) is used to describe an organism that is so small that, normally, it cannot be seen without the use of a microscope. Viruses, bacteria, fungi, protozoa and some algae are all included in this category.

Prokaryotes and eukaryotes

There are many differences between prokaryote and eukaryote cells. The main features are the presence of a nucleus, organelles, such as mitochondria and chloroplasts, and complex internal membranes in eukaryotes. Bacteria are prokaryotes; all other microbial cells are eukaryotes.

The importance of microbiology

Microbes are essential to life. Among their many roles, they are necessary for geochemical cycling and soil fertility. They are used to produce food as well as pharmaceutical and industrial compounds. On the negative side, they are the cause of many diseases of plants and animals and are responsible for the spoilage of food. Finally, microbes are used extensively in research laboratories to investigate cellular processes.

What is a microbe?

A **microbe** or **microorganism** is a member of a large, extremely diverse, group of organisms that are lumped together on the basis of one property – the fact that, normally, they are so small that they cannot be seen without the use of a microscope. The word is therefore used to describe **viruses**, **bacteria**, **fungi**, **protozoa** and some **algae**: the relative sizes and nature of these are shown in *Table 1*. However, there are a few exceptions, for example, the fruiting bodies of many fungi such as mushrooms are frequently visible to the naked eye; equally, some algae can grow to meters in length. Generally, microbes may be considered as fairly simple organisms. Most of the bacteria and protozoa and some of the algae and fungi are single-celled microorganisms, and even the multi-celled microbes do not have a great range of cell types. Viruses are not even cells, just genetic material surrounded by a protein coat, and are incapable of independent existence.

The science of microbiology did not start until the invention of the microscope in the mid 16th century and it was not until the late 17th century that Robert Hooke and Antoine van Leeuwenhoek made their first records of fungi, bacteria and protozoa. The late 19th century was the time when the first real breakthroughs on the role of microbes in the environment and medicine were made. Louis Pasteur disproved the theory of **spontaneous generation** (that living organisms spontaneously arose from inorganic material) and Robert Koch's development of **pure culture** techniques (see Topic D9) allowed him to show unequivocally that a bacterium was responsible for a particular disease. Since then the science has grown dramatically as microbiology impinges on all aspects of life and the environment.

Table 1. Types of microbes, their sizes and cell type

Microbe	Approximate range of sizes	Nature of cell	Section of book
Viruses	0.01–0.25 μm	Acellular	K
Bacteria	0.1–10 μm	Prokaryote	D,E,F
Fungi	2 μm–>1 m	Eukaryote	G,H
Protozoa	2–1000 μm	Eukaryote	J
Algae	1 μm–several meters	Eukaryote	I

Prokaryotes and eukaryotes

Within the microbial world can be found two different categories of cell type, **prokaryote** and **eukaryote**. Bacteria are prokaryotes: they lack a distinct nuclear membrane, the organelles associated with energy generation, such as mitochondria and chloroplasts, and complex internal membranes, such as endoplasmic reticulum and Golgi apparatus, which are found in eukaryotes. A comparison of the main features of these two categories of cell is shown in *Table 2*, but other differences do occur. Although the basic mechanisms of DNA replication (Topic C2), RNA synthesis (Topic C4) and protein synthesis (Topic C7) are the same in both prokaryotes and eukaryotes, there are differences in the components and enzymes involved. These are discussed in the appropriate topics.

The importance of microbiology

Microbes impinge on all aspects of life; just a few of these are listed below.

- **The environment.** Microbes are responsible for the cycling of carbon, nitrogen and phosphorus (geochemical cycles), all essential components of living organisms (Topic F1). They are found in association with plants in symbiotic relationships, maintain soil fertility and may also be used to clean up the environment of toxic compounds (bio-remediation; Topics H6 and I4). Some microbes are devastating plant pathogens (Topic H7), which destroy important food crops, but others may act as biological control agents against these diseases.
- **Medicine.** The disease-causing ability of some microbes such as smallpox (Variola virus; Topic K8), cholera (*Vibrio cholera* bacteria; Section F3) and malaria (*Plasmodium* protozoa, Topic J7) is well known. However, microorganisms have also provided us with the means of their control in the form of antibiotics (Topic F7) and other medically important drugs.
- **Food.** Microbes have been used for thousands of years, in many processes, to produce food, from brewing and wine making, through cheese production and bread making, to the manufacture of soy sauce (Topic F2). At the other end of the scale, microbes are responsible for food spoilage, and disease-causing microbes are frequently carried on food (Topic F5).
- **Biotechnology.** Traditionally microbes have been used to synthesize many important chemicals such as acetone and acetic acid (Topic F2). More recently, the advent of genetic engineering techniques has led to the cloning of pharmaceutically important polypeptides into microbes, which may then be produced on a large scale.
- **Research.** Microbes have been used extensively as model organisms for the investigation of biochemical and genetical processes as they are much easier to work with than more complex animals and plants. Millions of copies of

the same single cell can be produced in large numbers very quickly and at low cost to give plenty of homogeneous experimental material. An additional advantage is that most people have no ethical objections to experiments with these microorganisms.

Table 2. The major differences between prokaryote and eukaryote genetic and cellular organization

Prokaryotes	Eukaryotes
Organization of the genetic material and replication	
DNA free in the cytoplasm	DNA is contained with a membrane bound nucleus. A nucleolus is also present
Only one chromosome	>1 chromosome. Two copies of each chromosome may be present (diploid)
DNA associated with histone-like proteins	DNA complexed with histone proteins
May contain extrachromosomal elements called plasmids	Plasmids only found in yeast
Introns not found in mRNA	Introns found in all genes
Cell division by binary fission – asexual replication only	Cells divide by mitosis
Transfer of genetic information occurs by conjugation, transduction and transformation	Exchange of genetic information occurs during sexual reproduction. Meiosis leads to the production of haploid cells (gametes) which can fuse
Cellular organization	
Cytoplasmic membrane contains hopanoids. Lipopolysaccharides and teichoic acids found	Cytoplasmic membrane contains sterols
Energy metabolism associated with the cytoplasmic membrane	Mitochondria present in most cases
Photosynthesis associated with membrane systems and vesicles in cytoplasm	Chloroplasts present in algal and plant cells
	Internal membranes, endoplasmic reticulum and Golgi apparatus present associated with protein synthesis and targetting
	Membrane vesicles such as lysosomes and peroxisomes present
	Cytoskeleton of microtubules present
Flagella consist of one protein, flagellin	Flagella have a complex structure with 9+2 microtubular arrangement
Ribosomes – 70S	Ribosomes – 80S (mitochondrial and chloroplast ribosomes are 70S)
Peptidoglycan cell walls (eubacteria only: different polymers in archaebacteria)	Polysaccharide cell walls, where present, are generally either cellulose or chitin

B1 HETEROTROPHIC PATHWAYS

Key Notes

Nutritional types

Metabolism is divided into those pathways that are degradative (catabolic) and those that are involved in synthesis. Catabolic pathways often produce energy. Microbes that utilize organic molecules as a source of energy are called heterotrophs. Phototrophs obtain energy from light, and lithotrophs obtain energy from inorganic compounds.

Glycolysis

Most microbes utilize the glycolytic pathway for the catabolism of carbohydrates such as glucose and fructose. The products of this pathway are pyruvate, which can be further metabolized via the citric acid cycle, forming adenosine 5′-triphosphate (ATP) and the reduced form of nicotinamide adenine dinucleotide ($NADH + H^+$). This pathway is located in the cytoplasm of microbes and can function in the presence or absence of oxygen.

The Entner–Doudoroff pathway

The bacterial genera *Pseudomonas*, *Rhizobium* and *Agrobacter* substitute the Entner–Doudoroff pathway for the glycolytic pathway. This pathway is not as efficient in producing energy, with 1 mole of ATP being formed for each mole of glucose metabolized.

Pentose phosphate pathway

The pentose phosphate pathway produces $NADPH + H^+$ and sugars (4 C, 5 C). These are required for many synthetic reactions. When organisms are growing on a pentose (5 C) sugar, the pathway can be used to produce carbohydrates for cell-wall synthesis. Glyceraldehyde-3-phosphate formed by the pathway can be used to generate energy by glycolysis or by the Entner–Doudoroff pathway.

Citric acid cycle

The metabolism of pyruvate (formed by glycolysis) to CO_2 by the citric acid cycle is the major mechanism of ATP generation in the cell and is also an important source of carbon skeletons for biosynthesis. The fully functioning pathway requires oxygen; however, some organisms possess an incomplete cycle that can function in the presence or absence of oxygen but generates little or no energy.

Fermentations

$NADH + H^+$ produced by catabolic reactions such as the citric acid cycle can be oxidized by the electron-transport pathway in the presence of oxygen. However, in the absence of oxygen, many microbes utilize fermentation reactions to reoxidize $NADH + H^+$. Microbial fermentations are characterized by the end products formed. *Clostridia* are unusual in that they form ATP from the fermentation of amino acids by the Stickland reaction.

ATP yields

The citric acid cycle is the most efficient mechanism for generating ATP from glucose in the presence of oxygen. For microbes that live in environments where oxygen is absent or only present intermittently, ATP generation is less efficient.

Related topics

Bacterial taxonomy (D1)

Bacterial growth and cell cycle (D8)

Nutritional types

Metabolism in all cells is divided into **catabolic** (those pathways involved in breakdown of organic molecules for energy and the production of small compounds that may be used for synthesis) and **anabolic** (pathways involved in synthesis) processes. In all organisms these pathways are balanced as the energy required for anabolic processes is produced by catabolic pathways. In mammalian cells, energy production has been maximized by the use of oxygen and thus the cell is usually well supplied with energy; however, in microbes this is not always the case. Microbes can be divided into metabolic classes which relate to the sources of energy they use. The three groups are **heterotrophs** which utilize organic molecules as a source of energy (these are also called **chemo-organotrophs**), **phototrophs** which obtain energy from light, and **lithotrophs** which obtain energy from inorganic compounds. Carbon for cell synthesis is obtained from organic molecules; however, some microbes, including the phototrophs, fix CO_2.

Glycolysis

The **majority** of microbes utilize the **glycolytic pathway (**also known as the **Embden–Meyerhof pathway)** for the catabolism of carbohydrates such as glucose and fructose (*Fig. 1*). This series of reactions occurs in the cytoplasm of microbes and can operate either anerobically (in the absence of oxygen) or aerobically (in the presence of oxygen).

The overall equation for this pathway is

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i + 2\text{NAD}^+ \rightarrow 2 \text{ pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+$$

Pyruvate formed by glycolysis can be further metabolized in the presence of oxygen to generate energy via the **citric acid cycle** or can be used for synthesis of other compounds such as amino acids. Adenosine triphosphate (ATP) can be used directly to drive uptake of substrates or can be used to drive synthetic reactions. $NADH + H^+$ can be used to produce energy via **oxidative phosphorylation** (a method of ATP formation that requires electron transport; see Topic B2) or can be used as a source of H^+ for reduction reactions. Some organisms such as the bacteria *Clostridia* utilize **inorganic pyrophosphate** (PP_i) in place of ATP as a source of energy to drive the formation of pyruvate from phosphoenolpyruvate and for the conversion of fructose-6-phosphate into fructose-1,6-bisphosphate.

The Entner–Doudoroff pathway

A **minority** of bacteria including *Pseudomonas*, *Rhizobium* and *Agrobacter* substitute the **Entner–Doudoroff** pathway for the glycolytic pathway (*Fig. 2*). The pathway yields 1 mole each of ATP, $NADPH + H^+$ and $NADH + H^+$ for each mole of glucose **metabolized**. The products of this pathway, like those of glycolysis, can be used for a variety of functions; however, the $NADPH + H^+$ formed is used for synthetic reactions.

Pentose phosphate pathway

The **pentose phosphate pathway** or **hexose monophosphate pathway** may operate at the same time as glycolysis or the Entner–Doudoroff pathway. This pathway can also operate either in the presence or absence of oxygen. The pentose phosphate pathway is an important source of energy in many microorganisms; however, its major role would seem to be for biosynthesis. The basic outline of this pathway is shown in *Fig. 3*. The pathway produces $NADPH + H^+$