International Journal of Education and Science Research Review
E-ISSN 2348-6457
Volume-8, Issue-6 Nov-Dec- 2021
P-ISSN 2349-1817
www.ijesrr.org
Email- editor@ijesrr.org

Analytical Approaches in Studies on Intermediary Metabolism

Dr. Shiny Joy[1], Dr. K. Padmaja[2]

[1]Nagpur Veterinary College (MAFSU), Nagpur;
[2]College of Veterinary Science (SVVU), Tirupati

Corresponding author: Dr. Shiny Joy

Studying metabolism involves exploring the biochemical reactions and processes that convert food into energy and other essential molecules within cells. Here are some of several common approaches used to study metabolism.

1. Blood & Tissue Analysis

i. It is possible to obtain information relating to the metabolic activity of an organ by determining the quantities of a substance in the arterial blood to the organ and the venous blood from it (A V Difference). The Fick principle can be used to calculate the amount of blood flow to an organ by measuring the concentration of a marker substance in the arterial and venous blood, and the amount of the substance taken up by the organ over time (Joseph et al., 2020)

ii. Catheterization through the antecubital vein via the innominate vein and superior vena cava into the right atrium, right ventricle, coronary sinus or a pulmonary, renal or hepatic vein may be accomplished under fluoroscopic control

iii. This permits withdrawal of blood samples from various levels of the vascular system and determination of metabolic changes due to the function of different organs when the quantities of substances in the venous samples are compared with the values from samples of arterial blood.

iv. Utilization of oxygen, glucose and other substances by normal and failing hearts, the synthesis of hormones by the adrenal glands and other metabolic processes have been studied by the catheterization procedure (Sonavane et al., 2015). Quantitative estimation of tissue constituents can be studied this way.

2. Analysis of Excretions

The metabolism of various substances in the body produces characteristic end products which appears in the urine and at the times in the feces and analysis of these excretions often provides useful information.

Balance studies in which the total intake of a substance in the food and the total output of the same substance and/or its metabolic products in all excretions are measured. This permits construction of a balance sheet from which conclusion may be drawn relative to nature and level of metabolic activity of the substance. This approach has been valuable in studies of gross protein and mineral metabolism (Katyayan et al., 2020).

3. Respiration exchange

The oxidation of food stuffs in the body consumes oxygen and produce carbon dioxide. The volume relation of these gases is characteristic of the oxidation of glucose (carbohydrate), of protein (amino acid) and of fats (fatty acid and glycerol). The respiratory quotients CO_2/O_2 are

1.00 for Carbohydrates

0.80 for protein

0.707 for fats

If the protein metabolized in a given time by an animal is determined by urinary N_2 excretion and the O_2 consumption and CO_2 production are also measured, it is possible to calculate the quantities of Carbohydrate, fat and protein oxidized and the energy from each.

It must be remembered that respiratory quotient of different organs and tissues may differ considerably at any given time and the respiratory metabolism of the animal represents the additive effects of the metabolisms of the component tissues (Candell et al, 2020)

4. Removal of Endocrine gland and other organs

This approach to study metabolism has yielded much information.

Diabetic patients and animal made diabetic by pancreatectomy exhibit marked alterations of metabolic processes including hyperglycemia, glucosuria, ketonuria (increased blood ketone bodies: acetone, acetoacetic acid and β hydroxy–butyric acid) increased urinary N_2, low liver and muscle glycogen and low repiratory quotient. These symptoms are due to insulin deficiency which results in profound changes in the metabolism of glucose, fatty acids and amino acids. When completely diabetic animals are fasted, the blood sugar remains elevated; glucose and N_2 continue to be excreted in urine at a relatively constant rate for a considerable period of time. The excretion of ketone bodies also persists. Diabetic animal have been used to determine whether or not a substance forms glucose or acetoacetic acid in the body. (Shin et al., 2016)

It is possible to produce diabetic mellitus by administering Alloxan that destroy β cells of pancreas which forms insulin.

Animal with Phlorizin Diabetes has been used to study metabolism. When animals are given subcutaneous daily doses of glucoside phlorizin suspended in oil, the capacity of renal tubules to reabsorb glucose is destroyed which results that glucose pass rapidly through the kidney into the urine resulting hypoglycemia and glucosuria (Brouwers et al., 2013). Under these conditions, the tissues use little sugar and there is greatly increased rate of protein and fat metabolism with ketosis.

The removal of pituitary and adrenal glands produces marked changes in metabolism and is frequently resorted to in various types of studies. Injection of hormones into normal and operated animal is also used metabolic studies.

5. In Vivo and In Vitro Studies

Animal models are used to study how metabolism is regulated in response to interventions (Speakman, 2013). In vitro methods of studying metabolism consists in perfusing living organs in situ, or after removal from the body, with blood or other fluids and studying the changes in composition of the fluids and studying the changes in composition of the fluid after passage through the organs. Various substances may be added to the perfusing fluids and their chemical changes noted. (Thomas et al., 2000). Eg. When liver is perfused with alanine, the pyruvic acid of the perfusate is increased showing that the liver cells deaminize alanine and form pyruvic acid.

6. Warburg's Tissue Slice Technique

Fresh surviving tissues may be cut into thin slices, placed in appropriate media, such as Ringer's solution and used to study the chemical changes produced when certain substances are added. This preparation may also be placed in Barcroft Warburg manometric apparatus and their respiratory exchange may be studied (Elliott, 1955)

Eg. it can be shown that liver slices act upon a.a to deaminize them and form urea from NH_3 produced. It may also be possible to follow the metabolism of keto acids formed in the deamination process. The tissues slice technique provides one of the most valuable methods for metabolism studies.

Finely macerated suspensions of tissue (homogenates) instead of slices, occasionally are used for *in vitro* studies. Here, the cellular structures are broken down to liberate the enzyme systems. Such preparations do not so nearly simulate the metabolic process of intact tissues as do tissue slices in which most of the cells are intact.

7. Studies with purified enzyme system

A large proportion of modern biochemical research is based upon the use of isolated enzyme; in many cases highly purified. The use of purified enzyme permits kinetic and energetic characterization of the reaction it catalyzes (Lowry et al., 2015) through determination of rate and equilibrium constants.

Also, the cofactors required as part of the enzyme system, the inhibitory action of various substances, the effects of pH and temperature and the presence or absence of certain essential groups such as –SH in the enzyme may be determined.

But, it does not give an accurate picture as to how the reaction proceeds, is controlled and is related to other metabolic processes in the complex integrated environment of the living cell.

8. Inhibition of enzyme system with poisons

When one is working with a tissue or extract containing a number of enzymes, it is frequently possible to add a substance that selectively inactivates one or more of the enzymes, thereby permitting a better study of the action of those not inhibited (Gupta, 2016) This is particularly important when metabolism of a substance proceeds through a succession of reaction catalyzed by different enzymes.

Eg. Suppose a substance A is metabolized through changes such as

A----------> B----------->C----------> D

each catalysed by specific enzyme. Suppose we wish to study the formation of C but that C is very rapidly converted to D and does not accumulate in quantities that can be accurately determined. If a substance can be added that poisons the enzyme catalysing the conversion of C to D without inhibiting the other enzyme, then C will accumulate.

Eg. Muscle poisoned with iodoacetic acid converts glycogen to hexose phosphates but cannot carry out the breakdown further because essential enzymes have been inhibited.

9. Inborn Errors of Metabolism

There are number of metabolic abnormalities that are congenital, present throughout life and hereditary. Such abnormalities are represented by alkaptonuria, pentosuria, cystinuria, congenital porphyria, steatorrhea, albinism and galactosemia. In some of these conditions, failure of a metabolic step leads to the excretion of intermediate products which cannot be carried further along the metabolic path because of the specific enzyme deficiency but which the normal animal readily metabolizes (Rice et al 2018).

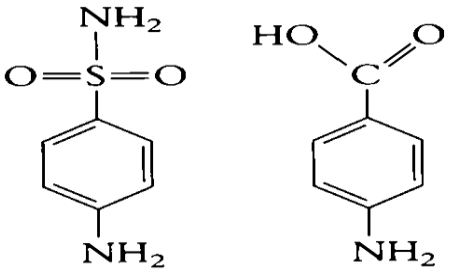
The identification of these intermediate metabolic products in the urine has to a limited extent aided in establishing the steps involved in the metabolism of a substance.

Eg. The classical example is the information relating to the metabolism of tyrosine and phenylalanine provided by individuals with alkaptonuria and phenylketonuria who excrete intermediate products of these amino acids in the urine.

10. Competitive analogue – metabolic inhibition

This method uses the use of substance similar to the structure of the substances required by the organism. These substances act by combining with and inactivating the enzyme system or systems for which the metabolite is a normal cofactor, thus creating a deficiency of the metabolite, with the resultant physiological changes (Moreno-Sánchez et al, 2008)

Eg. The bacteriostatic action of sulfanilamide upon microorganisms is due to the fact that it is a structural analogue of para-amino-benzoic acid which is necessary cofactor for bacterial enzyme systems. The sulfanilamide is 'competitive inhibitor'



Sulfanilamide PABA

The substance pyrithiamine is an analogue to Vitamin Thiamine and when it is given to mice, it causes typical symptoms of thiamine deficiency because it displaces thiamine in essential enzyme systems.

When an analogue of a metabolite produces metabolite deficiency symptoms that are reversed by giving metabolite, it is logical to conclude that the metabolite probably functions as a cofactor in one or more enzyme systems.

11. Use of radioactive and stable isotopes as tracer atoms

When a molecule of fatty acid, amino acid, sugar, phosphate or other food substance is taken into the body, it become mixed with large number of other like molecules in the 'metabolic pool' and it is impossible by ordinary methods to trace the chemical pathway of its metabolism.

Using phenyl group, Knoop developed β-oxidation in fatty acid metabolism (Houten and Wanders, 2008) This was first successful use of tracers in metabolic studies. The objection to this study was that introduction of phenyl group may change the chemical properties of the acids so that they are not oxidized as are the natural unsubstituted acids.

The ideal way of tagging a molecule for metabolic studies would be to substitute into its isotopic atoms which have same chemical properties as the atoms replaced and also possess properties by which it is easily detected in the various compounds formed in the metabolic reactions (Wilkinson, 2018).

The radioactive isotopes may be easily determined by instruments such as the Geiger Muller Radiation Counter and Proportional and Scintillation Counters. Heavy and light isotopes which are not radioactive are determined with mass spectrometer. Isotopic tracer compounds permit the study of not only chemical pathways in metabolism but also the rate of change or turnover of a substance in the body (Katz & Wood, 1963)

In recent years, large number of radioactive and heavy and light isotopes of the elements have been produced artificially through bombardment of atomic nuclei with proton $_1H^1$, deuteron $_1H^2$, α particles, $_2He^4$,

and neutrons ${}_0n^1$. Neutrons enter the nuclei of bombarded atoms most readily because being electrically neutral, they are not repelled by the positively charged nucleus as are protons deuterons and α particles.

By integrating these various approaches, researchers can gain a comprehensive understanding of metabolic processes and their regulation.

References:

Brouwers B, Pruniau VPEG, Cauwelier EJG, Schuit F, Lerut E, Ectors N, Declercq J, Creemers JWM. Phlorizin pretreatment reduces acute renal toxicity in a mouse model for diabetic nephropathy. J Biol Chem. 2013 Sep 20;288(38):27200-27207. doi: 10.1074/jbc.M113.469486.

Candell L. M., Shaw G. A., and Thompson K. J. Model and Personal Sensor for Metabolic Tracking and Optimization 2020; Lincoln Laboratory Journal 24 (1) 114-140

Elliott K.A.C., Tissue slice technique, Methods in Enzymology, Academic Press, Volume 1, 1955, Pages 3-9, ISSN 0076-6879, ISBN 9780121818012, https://doi.org/10.1016/0076-6879(55)01005-7.

Gupta P.K., Chapter 8 - Biotransformation, Editor(s): P.K. Gupta, Fundamentals of Toxicology, Academic Press, 2016, Pages 73-85, ISBN 9780128054260, https://doi.org/10.1016/B978-0-12-805426-0.00008-1.

Houten SM, Wanders RJ. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. J Inherit Metab Dis. 2010 Oct;33(5):469-77. doi: 10.1007/s10545-010-9061-2.

Joseph P, Oliveira RKF, Eslam RB, Agarwal M, Waxman AB, Systrom DM. Fick principle and exercise pulmonary hemodynamic determinants of the six-minute walk distance in pulmonary hypertension. Pulm Circ. 2020 Sep 11;10(3):2045894020957576. doi: 10.1177/2045894020957576.

Katyayan K., Yi P.., Cassidy K. Excretion Mass Balance, and Metabolism of [14C] LY3202626 in Humans: An interplay of microbial reduction, reabsorption and aldehyde Oxidase Oxidation that leads to an extended excretion profile. Drug Metabolism and Disposition 2020; 48 (8) 698-707. DOI: https://doi.org/10.1124/dmd.120.000009

Katz, J., & Wood, H. G. (1963). The use of C14O2 yields from glucose labeled with C14 to determine the pathways of glucose metabolism. The Journal of Biological Chemistry, 238, 517-523.

Lowry B, Walsh CT, Khosla C. In Vitro Reconstitution of Metabolic Pathways: Insights into Nature's Chemical Logic. Synlett. 2015;26(8):1008-1025. doi: 10.1055/s-0034-1380264.

Moreno-Sánchez R., Saavedra E., Rodríguez-Enríquez S., Olín-Sandoval V., Metabolic Control Analysis: A Tool for Designing Strategies to Manipulate Metabolic Pathways, BioMed Research International, 2008, 597913, 30 pages, 2008. https://doi.org/10.1155/2008/597913

Rice G. M. and Steiner R. D., Inborn Errors of Metabolism 2018 Pediatrics in Review 37(1):3

Shin, S. J., Chung, S., Kim, S. J., Lee, M., Yoo, H., Kim, W., Ahn, B., Kim, S., Moon, D., Kim, J., & Ko, H. (2016). Effect of Sodium-Glucose Co-Transporter 2 Inhibitor, Dapagliflozin, on Renal Renin-Angiotensin System in an Animal Model of Type 2 Diabetes. PLOS ONE, 11(11), e0165703. https://doi.org/10.1371/journal.pone.0165703

Sonavane S. K., Milner D. M., Singh S. P., Aal A. K. A., Shahir K. S. and Chaturvedi A. Comrehensive imaging review of the superior vena cava. Radiographics 2015; 35(7) doi10.1148/rg.2015150056.Speakman, J. R. (2013). Measuring energy metabolism in the mouse – theoretical, practical, and analytical considerations. Frontiers in Physiology, 4, 34.

Thomas DL, Lythgoe MF, Pell GS, Calamante F, Ordidge RJ (2000). "The measurement of diffusion and perfusion in biological systems using magnetic resonance imaging". Phys Med Biol. 45 (8): R97-138. doi:10.1088/0031-9155/45/8/201

Wilkinson DJ. Historical and contemporary stable isotope tracer approaches to studying mammalian protein metabolism. Mass Spectrom Rev. 2018 Jan;37(1):57-80. doi: 10.1002/mas.21507.

*****