Hepatic Protein Metabolism and Amino Acids in Nitrogen Balance
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The liver is a major producer of proteins not only for its own central role in energy production and storage, but in the production of the most abundant extracellular protein in the body – Albumin. Therefore any disease process which affects the liver can have profound consequences for energy production and storage and subsequently body mass. Loss of albumin production can have significant effects in the compartmentalisation of body water due to it being the major force in the maintenance of osmotic pressure in the ECF.

Protein turnover refers to the continuous degradation and re-synthesis of all cellular proteins.

Adults turn over 1-2% of their total body protein each day, with the equivalent of 20-30g of protein (in the form of Nitrogen) excreted each day. 70-80% of liberated amino acids are re-utilized into proteins with 20-25% turned into urea for excretion.

The rate of protein turnover is very variable and (of the systems studied so far) reflects usage/demand. Therefore high rates are found in tissues undergoing structural re-arrangement e.g. uterine tissue during pregnancy, tissue damaged as a result of trauma, skeletal muscle during starvation. Severe Burns are complicated by the fact that there is not just extensive tissue damage (and therefore attempts at re-modelling) but also by the fact that significant amounts of protein can be lost in the exudate from the damaged tissue. (Blisters are so firm because of the high osmotic pressure brought about by the presence of protein in the contained fluid)

Half-Life of Proteins

Liver proteins half-lives range from the short acting <30 minute ones to those of >150 hours.

To carry out their functions effectively proteins in cells have to be in the right place at the right time and in the right concentration. If this fails then it has profound consequences for cell division, gene expression and the development of cancer.

Degradation can be divided into Lysosomal and Non-lysosomal, with the former being carried out in the reticulo-endothelial system of the liver. This comprises the sinusoidal endothelial cells, Kupffer cells and pit cells. Sinusoidal endothelial cells remove soluble proteins and fragments through the fenestrations known as sieve plates on their luminal surface. They are important for removing fibrin and fibrin degradation products, collagen and IgG complexes. Kupffer cells are the liver resident macrophages and perform a similar function, but phagocytose particulate matter.
Non-lysosomal degradation

Degradation is a selective process with the lifetime of a protein being regulated by specialized proteolytic systems. Rapidly degraded proteins include those that are defective because of incorrect amino acid sequences or because of damage during normal function. Rapid turnover of key metabolic enzymes is only part of the story. Transcription factors and the cyclins (involved in the regulation of the cell cycle) are two families for which one can easily see good reasons for turning off quickly.

PEST sequences – target proteins for degradation Proline (P) glutamic acid (E) serine (S) threonine (T) are hydrophilic regions containing at least one of each in a total of 12 amino acids. These regions are probably conditional – ie they only become exposed during catalytic activity (or whatever) and may explain the readiness of degradation in proteins which have mutations. There are other proteolytic sequences being discovered but PEST is the oldest (1986 when first mooted, evidence for their existence and characterisation in the 1990’s). The other region studied is on the cyclins – the impressively named cyclin destruction box!!

Amino terminal sequences may also play a role in the determination of half-life. The amino acids Met, Gly, Ala, Ser, Thr and Val appear to confer a stabilising influence and are found in proteins with a half-life greater than 20hr.

In eukaryotes this degradation is mediated by a 76 aa protein called ubiquitin. – so named because of it’s impressive conservation throughout nature, basically found all animals that swim, fly, walk or crawl.

Ubiquitin is covalently linked to protein slated for destruction via a 3 stage ATP dependent enzymatic pathway. This initial attachment seems to flag up a further series of ubiquitinations.

Polyubiquitins seem to be the recognition site for the cellular executioner – the proteosome. Proteosomes are made up of a central cylinder – where the degradation takes place possibly at several sites – and two regulatory ‘lids’. It is these lids which bind the polyubiquinated protein, unfolds them and then feeds them into the cylinder for hydrolytic degradation.

Amino acids released by this process are re-cycled into new proteins. Those that aren’t are rapidly degraded via the urea cycle. Excess amino acids are not stored. Regardless of source, those which aren’t immediately incorporated into proteins are degraded. Consumption of excess amino acids thus serves no purpose that cannot
Rate of Degradation of Protein

Phosphorylation

Post-translational phosphorylation is one of the most common protein modifications that occurs in animal cells. The vast majority of phosphorylations occur as a mechanism to regulate the biological activity of a protein and as such are transient. In other words a phosphate (or more than one in many cases) is added and later removed. Physiologically relevant examples are the phosphorylations that occur in glycogen synthase and glycogen phosphorylase in hepatocytes in response to glucagon release from the pancreas. Phosphorylation of synthase inhibits its activity, whereas, the activity of phosphorylase is increased. These two events lead to increased hepatic glucose delivery to the blood.

The enzymes that phosphorylate proteins are termed kinases and those that remove phosphates are termed phosphatases. Protein kinases catalyze reactions of the following type: $\text{ATP + protein } \leftrightarrow \text{ phosphoprotein + ADP}$

In animal cells serine, threonine and tyrosine are the amino acids subject to phosphorylation. The largest group of kinases are those that phosphorylate either serines or threonines and as such are termed serine/threonine kinases. The ratio of phosphorylation of the three different amino acids is approximately 1000/100/1 for serine/threonine/tyrosine. Although the level of tyrosine phosphorylation is minor, the importance of phosphorylation of this amino acid is profound. As an example, the activity of numerous growth factor receptors is controlled by tyrosine phosphorylation.

Prenylation

A number of proteins are isoprenylated which alters their biological activity. Isoprenoid groups are either 15 or 20 carbon atom derivatives from cholesterol biosynthesis. They are mainly concerned with the modification of signalling proteins like ras and so may there be a role for inhibiting this posttranslational modification in cancer chemotherapy.
3-stage ubiquitination.

Nitrogen Balance

As the name suggests this is a measure of the difference between what goes in and what comes out and therefore is generally referred to as either Positive or Negative nitrogen balance, with the assumption being that people who are healthy are in nitrogen equilibrium.

Input – great bulk of incoming entering the typical animal does so in the form of α-amino acids from proteins in the diets. There are about 300 amino acids in nature, of which 20 are found in proteins and 8 or 9 are designated as essential – meaning that they can’t be synthesised and have to be supplied in the diet.

Output – the basic form of output is the NH₃ molecule. However, since this is toxic most species convert it into a non-toxic excretory product. Depending on whether you live on land or in water will depend on which form you excrete.

Positive Nitrogen Balance

Pregnancy is probably the most common instance of positive nitrogen balance. Recommended
daily intake to remain in nitrogen balance is 0.8g/Kg\(^{-1}\) body weight in an adult. In pregnancy this rises to 1.3 g/Kg\(^{-1}\) body weight. In the first few months of life the daily intake must be in the order of 2.4 g/Kg\(^{-1}\) body weight. (i.e. 3 times that of an adult).

Body builders are another example of positive nitrogen balance, but only because they take anabolic steroids.

Patients recovering from illness or surgery go into a phase of positive nitrogen balance

**Negative Nitrogen Balance**

Malnutrition is the obvious too common example of negative nitrogen balance. The WHO estimate that around 49% of the annual 10.4 million deaths in children under 5 years in developing countries is a direct result of malnutrition i.e. over 5 million children each year.

There are two basic forms:–

- Marasmus which is characterised by severe wasting simply as a result of insufficient calorie intake – not simply protein deficiency. Anorexics, cancer cachexia as well as children in Africa.

  - Characteristic features:–
    - <60% of normal body weight for age and sex
    - loss of somatic protein
    - loss of muscle and fat in the extremities
    - head appears to be ‘large’ in relation to the rest of the body (especially true in children)

- Kwashiokor where there is swelling of the abdomen and arms and legs. This is caused by insufficient protein in the diet. Caloric intake is adequate, but protein not. This occurs when the principal dietary component is maize.

  - Characteristic features:–
    - loss of visceral protein
    - oedema
    - fatty liver
    - immune deficiency

Apart from the lack of an adequate diet, disease states in which increased catabolism is present can also lead to negative nitrogen balance

- Multiple trauma or extensive trauma where there is a lot of tissue damage and subsequent remodelling. In burns patients the tissue damage/remodelling is complicated further by the fact that the exudate contains protein that is effectively ‘lost’ to the body so we have the situation where there is both an increased metabolic requirement for protein at the same time as an increase in the rate of loss.

  - Head injury is another state in which negative nitrogen balance develops, despite the fact that the tissue damage can be relatively minor in quantitative terms. The average nitrogen loss in a severely brain-injured patients can be double or triple that of a normal patient.

  - Comparatively speaking head injury patients have a metabolic response similar to that of patients with burns of 20-40% of the body surface. This rate of loss will produce a 10% decrease in lean mass within 7 days, underfeeding these patients for 2-3
weeks could result in total weight loss in the order of 30%. A complicating factor is that it is difficult to put these patients back into positive nitrogen balance it appears that the more nitrogen that gets put in the more comes out. Causes of this state appear to be multifactorial partly adrenergic, partly secondary to the head injury e.g. temperature control, muscle tone.