Protein synthesis in the Liver and the Urea Cycle

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This lecture will consider the features of how nitrogen is removed from amino acids and converted to urea and the major proteins synthesised by the liver.

In this overview we can see how the amino acid pool is added to from dietary protein and how the intracellular proteins circulate these free amino acids in a continuous cycle of synthesis and breakdown. Excess amino acids are metabolised (not stored for use as potential energy because this can be done more efficiently using other energy sources). The carbon skeleton, as an \( \alpha \)-keto acid, is fed into the citric acid cycle to be incorporated into glucose production whilst the ammonia is largely excreted, although some is used in the biosynthesis of amine containing substances.

In the liver; input of amine groups comes from dietary amino acids, alanine from muscle and glutamine from muscle. The \( \alpha \)-ketoglutarate / glutamine pair are the most common of the donor /acceptor pairs. Serum levels of the aminotransferase enzymes are used clinically as indicators of liver cell damage (these reactions occur in the hepatocytes) – the most clinically useful being Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – higher levels indicating that more enzyme has leaked from the damaged hepatocyte.

(a) \( \alpha \) ketoglutarate acting as an amine acceptor giving us L-glutamate and an \( \alpha \) keto acid. PLP represents pyridoxal phosphate, which is the metabolically active form of Vitamin B6 and is a co-factor in all of these reactions.

(b)
In the bottom reaction alanine is deaminated to pyruvate - part of the glucose alanine cycle.

Glutamate Dehydrogenase reaction

This reaction goes both ways using NAD in the forward reaction and NADP the other way. The forward reaction generates α-ketoglutarate which is fed into the citric acid cycle and so hepatocytes are capable of upregulating GDH activity at times of energy depletion (at a cellular level at least). So as the illustration shows, ADP / GDP drive the reaction forward – because they represent the ‘low energy’ i.e. ATP or GTP have been ‘used’ by the cell so in order to reconstitute them substrate for the citric acid cycle is generated and ATP is replenished. When ATP concentration is high then glutamate is formed and because it is an amino acid, it is available for incorporation into protein.

Glutamine Synthetase reaction

A reaction which produces an amino acid – glutamine - suitable for incorporation into proteins. Its principal role appears to be as a circulating ammonia carrier, consequently it is the most abundant amino acid found in blood. In this state it is a neutral, non-toxic compound which passes readily through cell membranes.

Glutaminase

In most land animals glutamine is carried in the blood to the liver. As is the case for glutamate, the amino nitrogen is released only within the mitochondria by this enzyme glutaminase.

Glutamine synthetase is a cytosolic enzyme whereas glutaminase is a mitochondrial enzyme, so they are located in separate compartments which ensures that the liver is neither a net consumer or producer of glutamine. The differences in cellular location of these two enzymes allows the liver to scavenge ammonia that hasn’t been incorporated into urea and so ammonia concentration is controlled by either incorporation into urea or glutamine.
Glucose alanine cycle

In muscle, alanine is the principal ammonia scavenger and transporter. Glutamate collects the ammonia, the enzyme alanine aminotransferase (ALT) transaminates the amino group from glutamate, forming α-ketoglutarate, and the amino group gets attached to pyruvate, formed from glycolysis, making alanine. This gets transported in the blood, taken up by the liver where the reverse reaction occurs and the ammonia gets converted to urea. Pyruvate is recycled into glucose.

This is a superb illustration of economy of effort in solving two problems with one cycle. Moving carbon atoms of pyruvate, as well as excess ammonia, from muscle to liver as alanine. Then in the liver, alanine yielding pyruvate — the starting block for gluconeogenesis, and releasing ammonia for conversion into urea. The energetic burden of gluconeogenesis being imposed on the liver rather than muscle, so that muscle ATP can be devoted to muscle contraction.

The urea cycle.

Discovered by Hans Krebs and Kurt Henseleit in 1932. Henseleit was a medical student. Krebs came to Sheffield via Cambridge the following year.

The essential features of the urea cycle reactions and their metabolic regulation are as follows:-

Arginine either from the diet or protein breakdown, is cleaved by arginase generating urea and ornithine. In subsequent reactions a
new urea is built on the ornithine (from ammonia and \( \text{CO}_2 \)) making citrulline. This, in turn, is reconfigured into arginine. The enzymes responsible for this are found partly in the mitochondria and partly in the cytosol (like glutaminase/glutamine synthase).

The reactions of one turn of the cycle consume 3 ATP equivalents and a total of 4 high energy nucleotide \( \text{PO}_4^= \). Urea is the only compound generated by the cycle: all other components are re-cycled. The energy consumed by urea production is generated in the production of the cycle intermediates.

Control of the cycle is via up or down regulation of the enzymes responsible for urea formation. So with long term changes in the quantity of dietary protein, upregulation in the order of 20 times has been demonstrated. This can be due to either increased intake as with body builders – high protein low fat diets - or in starvation because muscle proteins are being broken down with the amino acid carbon skeletons providing the energy. Thus the amount of ammonia that must be excreted increases.

Defects in the urea cycle.

Absence of any of the enzymes involved in urea synthesis is not compatible with life. Deficiencies in any one of them can occur and are well described in the textbooks, but in terms of their likely impact on your future clinical practice they are insignificant and will probably be dealt with in your paediatrics modules. The common thread to them all is the elevation of ammonia levels in the blood.

![Diagram of the urea cycle](image)

Neurotoxicity associated with Ammonia.

Elevated blood ammonia is seen in severe liver disease, whether it be as a result of liver failure due to infection, toxicity or substantial surgical resection. This is something that is seen in the clinical practice (not uncommonly) and since ammonia is neurotoxic, is one of those things that staff are conscious of when a patient with liver disease becomes confused or comatose.

The mechanism for the increase in ammonia is basically the same in all cases. In simple terms, the blood doesn’t get exposed to enough liver parenchymal cells to have the ammonia removed. This can be due either to the fact that there simply aren’t enough living, metabolising cells because they’ve been killed off by the disease process be it viral or toxic. Or because, in cirrhosis for example, the resistance to blood flow through the liver is so great (because of fibrosis) that the blood by-passes the liver by flowing through large collateral veins. It therefore gets delivered to the brain directly.
Ammonia crosses the blood-brain barrier readily. Once inside it is converted to glutamate via glutamate dehydrogenase and so depletes the brain of $\alpha$ ketoglutarate. As ketoglutarate falls, so does oxaloacetate and ultimately citric acid cycle activity stops, leading to irreparable cell damage and neural cell death.

Major proteins produced by the liver which have significant extra-hepatic roles.

Albumin

A highly soluble, single polypeptide protein with a MW of 66000. Around 9-12 g produced per day with the rate of production being controlled by changes in colloid osmotic pressure and osmolality of the extravascular liver space. Production can be increased by 2 to 3 fold when necessary.

Albumin leaves the circulation via the interstitium to the lymph system and back to the circulation via the thoracic duct. Between 4-5% of total intravascular albumin extravasates per hour. This rate of movement is known as the Transcapillary Escape rate and is determined by :-
1. Capillary and interstitial free albumin concentration.
2. Capillary permeability to albumin
3. Movement of solute/solvent
4. Electrical charges across the capillary wall (albumin has a strongly negative charge)

The biological half-life in the circulation is around 16-18 hours

Functions

1. Binding and transport

There are 4 binding sites on albumin which have varying specificity for different substances. Competitive binding of drugs may occur at either the same site or different sites (causing conformational changes which affect other binding sites). The drugs that are important for albumin binding are; warfarin, NSAIDS, midazolam, thiopentone.

2. Maintenance of colloid osmotic pressure

Colloid osmotic pressure is the term used to describe the effective osmotic pressure across blood vessel walls which are permeable to electrolytes but not larger molecules. It is almost entirely due to plasma proteins.
The Starling equation

Net Driving Pressure = Kf x [(Pc – Pi) – rc(pc – pi)]
Kf is the filtration coefficient
Pc – hydrostatic pressure in the capillary
Pi – hydrostatic pressure in the interstitium
rc – the reflection coefficient is a correction factor used to correct the magnitude of the measured gradient to take account of the ‘effective oncotic pressure’ (ie in systems where protein concs are low e.g.CSF the rc will be close to 1 whereas in the liver lymph the conc of protein is high and the value is close to 0
pc – oncotic pressure in the capillary
pi – oncotic pressure in the interstitium.

So net fluid flux is proportional to this driving pressure. Also, the capillary hydrostatic pressure falls along the capillary from the arteriolar to the venous end and so the driving pressure decreases.

3. Free Radicals

Albumin has a large number of sulphhydryl groups. These thiols are able to scavenge free radicals – nitrogen and oxygen species. This may be particularly important in sepsis.

4. Anticoagulant effects.

Albumin has both anticoagulant and antithrombotic effects, both of which are poorly understood. They may be related to its binding of nitric oxide radicals which would have the effect of inhibiting inactivation and therefore prolonging the biological half – lives.

What causes albumin to decrease?

1. Decreased synthesis.

In liver disease or in large resections, the functional mass of the liver is reduced and therefore its ability to synthesise proteins is likewise reduced.

2. Increased catabolism

Very slow decline in levels because it is synthesised at such a rate and synthesis can be upregulated several fold.

3. Increased loss

* Nephrotic syndrome – where there is increased glomerular permeability which allows proteins to filter through and so loss of up to several grams of protein per day can occur
* Exudative loss in burns. Extensive tissue damage with concomittent damage to the capillaries and therefore loss of protein through the wall.
* Haemorrhage
* Gut loss

A rare syndrome – protein losing enteropathy in which the wall of the gut is unusually permeable to large molecules. More common however is ulcerative colitis where the site of ulceration is the site of increased permeability
Consequences of decreased serum albumin

1. Decreased colloid oncotic pressure

Decreased colloid oncotic pressure and oedema formation (oedema being the accumulation of fluid in the interstitial space). The formation of oedema is determined by the rate of fluid flux and the clearance of fluid by the lymphatics.

In critical illness there is increased leakage of albumin and decreased synthesis. There is a stronger correlation between colloid oncotic pressure and total protein because the decreased albumin synthesis is compensated for by increased synthesis of acute phase proteins.

2. Decreased ligand binding

Drug kinetics are altered, similarly hormone transport can be affected.

Disease processes associated with low serum albumin

Malnutrition – a diet with a high proportion of low-grade cereal proteins (such as maize) can lead to a deficiency in the essential amino acid lysine which in turn leads to a decrease in protein synthesis. In contrast starvation does not lead to low albumin levels – protein from muscle is used as the energy source.

Liver disease

Renal diseases – albumin loss through the glomerulus and to a small extent during dialysis.

Pre-eclampsia – the association of hypertension, proteinuria and oedema in pregnancy- there is a paradoxical decrease in plasma volume and capillary leak syndrome.

Burns / Trauma

Loss through leaky capillaries although burns patients can also develop a protein losing enteropathy

Sepsis

Increased capillary permeability – possibly due to bacterial endotoxins and cytotoxic T cells. There is also a profound reduction in plasma albumin associated with marked fluid shifts.

There is controversy about the use of albumin in clinical practice. Previously studies have shown a correlation between low serum albumin and mortality. Therefore the obvious thing to do, it would seem, would be to raise serum albumin levels by albumin infusion. However, these ‘normalisation’ regimes have not proved to be effective – in fact one meta-analysis has suggested a higher mortality rate in critically ill patients treated with albumin. It is still used by some – others adopt the “treat the reason for the capillary leakage and the patient will get better” strategy.

Clotting proteins produced by the liver
With the exception of von Willebrand Factor (VIII) the liver is responsible for the production of all of the coagulation proteins. Many are serine proteases which require activation.

Clotting cascade

In this example Factor 9a (a serine protease itself) activates Factor 10 by cleavage of the heavy chain.

Many have a γ carboxyglutamic acid residue which requires Vitamin K (also stored in the liver) for conversion.