EXECUTIVE SUMMARY

Interaction between water balance and protein metabolism of ruminants

Urea metabolism and acid/base homeostasis in small ruminants

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HYPOTHESIS

Nitrogen balance of an arid-adapted animal such as a sheep will be influenced by the water balance status of that animal. Urea is retained by the kidney during dehydration. Some of this urea will be recycled to the rumen, where it will contribute to the synthesis of microbial protein. This protein may be digested in the small intestine and will contribute to the protein uptake of the host animal. Alternatively, the urea may be broken down in the large intestine to ammonia, which may be returned via the portal circulation to the liver, where it may be re-synthesised directly to amino acids.

In the liver, metabolic pathways are embedded into a sophisticated structural and functional organisation with metabolic interactions between different hepatocyte populations. Metabolic zonation of N-metabolism is strict, i.e. urea synthesis enzymes and glutaminase (+ enzymes of gluconeogenesis) are present in periportal hepatocytes, whereas glutamine synthetase is found only in perivenous hepatocytes. The use of *in vitro* perfusion of the isolated liver to investigate these pathways is well established in rats.

This perfusion technique has led to the following hypothesis. The liver plays a major role in acid-base balance homeostasis. Both the urea cycle and the glutamine synthetase systems are sensitive to plasma pH; an acidosis repressing urea synthesis and increasing glutamine production. This raises levels of bicarbonate in the blood and increases export of glutamine to the kidney for proton excretion. An alkalosis causes the reverse to occur.

If this system is also operational in sheep, then the production of urea in the liver will be influenced by such diverse factors as dietary regime and water intake. There is to the best of our knowledge, no documented evidence of

(i) the zonation of N-metabolising pathways in sheep;

(ii) the effect of acid/base balance on the synthesis of urea in sheep.

(iii) the effect of plasma osmolarity on urea metabolism in the liver of sheep.

Experimental Procedures

SA Mutton Merino wethers, about 3 months old, are housed in indoor pens. All sheep are clinically examined on a regular basis, and mass changes determined weekly. Each sheep is anaesthetized with pentobarbitone via a jugular catheter, the left flank shaved, and a paracostal incision made about 3 cm caudal to the last rib, starting at the lumbar margin and extending to beyond the ventral midline. The abdominal organs are retracted so as to facilitate access to the liver. Suitable snares are placed around the combined aorta and vena cava anterior and posterior to the liver, as well as around the portal vein. A cannula is inserted via the portal vein into the branch leading to the small, dorsal lobe, and tied into the vein. Once perfusion with a heparinised, well-oxygenated physiological buffer at 39° C is commenced, the sheep is euthenased, the snares tied off and the liver immediately removed. A second cannula is tied into the vessel draining this lobe into the vena cava. The lobe is then transferred to the perfusion apparatus, and the experiment started.

Summary of Results:

Trial 1: Heterogeneity of hepatocytes

Changeover ante-/retrograde perfusion of 4 sheep livers using the modified Krebs/Ringer buffer with and without NH_4Cl was used to test this hypothesis. Ammonia removed by the liver in the antegrade direction was higher than that in the retrograde direction, as in the rat. Results supported the hypothesis that the hepatocytes in the sheep are indeed differentially distributed along the lobule.

Trial 2: Presence of glutamine/glutamate system in perivenous hepatocytes

Following treatment with sulfoximine (inhibition of glutamine synthetase), changeover ante-/retrograde perfusion of 4 sheep livers using the modified Krebs/Ringer buffer with and without NH_4Cl. The effluent was analysed *inter alia* for changes in urea and glutamine concentrations. The data supported the hypothesis that the glutamine system is largely confined to the perivenous system.

Trial 3: Presence of Urea cycle in periportal hepatocytes

Changeover ante-/retrograde perfusion of 4 sheep livers using HEPES buffer (inhibition of carbamoyl phosphate synthetase) with and without NH_4Cl. The effluent was analysed *inter alia* for changes in urea and glutamine concentrations. No evidence could be found of urea cycle zonation.

Trial 4: Effect of pH (acid-base balance) on Urea/Glutamine synthesis

. The acid-base status of the perfusate did not appear to affect the extent of the removal of ammonia from the inflow, unlike the rat. Furthermore, urea production is insensitive to pH changes, as was glutamine synthesis.

Importance of Results

This data provides the physiological and biochemical basis underlying the ammonia and amino acid metabolism of the ruminant liver. Many of the empirical results obtained from typical feeding trials remain unexplained, largely due to ignorance of the pathways (and their control) responsible for the uptake and metabolism of the ammonia, amino acids and peptides in the portal input to the liver. This model will expand out basic understanding of these processes, and in the long term allow us to better plan feeding strategies for our livestock.

Future Extension of this Programme

Since this research now falls outside the mandate of the Protein research Trust, it was decided to seek funding elsewhere for the continuation of this work. However, another aspect of the protein metabolism of the ruminant that also forms part of our Departmental research programme is the recycling of urea by the ruminant, particularly when fed a diet low in protein. I have made a new application for funding in this area.

Publications

Aside from numerous congress presentations:

1. SILOVE, M., BOOMKER, E.A. & VAN DER WALT, J.G. (1995). *In vitro* liver perfusion: a viable tool for the study of ruminant hepatic physiology. *Annual Congress of the Physiology Society of Southern Africa*, 4-6 September, Johannesburg
2. SILOVE, M., BOOMKER, E.A., VAN DER WALT, J.G. & KRIEK, N.P.J. (1995). *In vitro* liver perfusion: a viable tool for the study of ruminant hepatic physiology. *12th Faculty Day (75th Anniversary Symposium), Veterinary Science*, University of Pretoria, 27-29 September, Onderstepoort.
3. ROSSOUW, H.C., VAN DER WALT, J.G. & NELL, M. (1997). Zonation of the urea cycle and glutamine synthesis in the ovine liver. *25th Annual Congress of the Physiological Society of Southern Africa*, and the *2nd International Congress of the African Association of Physiological Sciences*, International Convention Centre, 21-24 September, Durban.

4. ROSSOUW, H.C., VAN DER WALT, J.G. & NELL, M. (1997). Zonation of the urea cycle and glutamine synthesis in the ovine liver. *14th Faculty Day, Veterinary Science*, University of Pretoria, 26 September, Onderstepoort.

5. ALI, A.M., ROSSOUW, H.C. & VAN DER WALT, J.G. (2000). Liver perfusion with hyperosmotic media stimulates glutamine and urea synthesis. *3rd International Congress of the African Association of Physiological Sciences*, 3-8 September 2000, University of Pretoria.

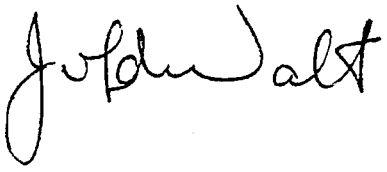
There have been two full papers that have flowed out of this work:

1. ROSSOUW, H.C., NELL, M.J., MOHAMED ALI, A. & VAN DER WALT, J.G. (1999). Ammonia partitioning between urea and glutamine in the perfused sheep liver: role of extracellular pH. *S.Afr.J.Anim.Sci.*, **29**(ISRP), 246-247.

2. ALI, A.M., ROSSOUW H.C., SILOVE, M. AND VAN DER WALT, J.G. (2000). Development of an improved technique for perfusion of the isolated caudal lobe of sheep liver. *Experimental Physiology*, **85**, 469-478.

As well as a chapter in a book:

3. LOBLEY, G.E., MILANO, G.D. & VAN DER WALT, J.G. The liver: Integrator of nitrogen metabolism. **Invited paper** at the International Symposium on Ruminant Physiology, 17-22 October, 1999, Pretoria, South Africa. Published as a chapter in "*Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*", edited by Cronjé PB, Boomker EA, Henning PH, Schultheiss W, van der Walt, JG, pp149-168. Published by CABI, Publishing, Wallingford, UK in 2000.



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