

Regulation of blood ammonia

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SUMMARY Disturbance of ammonia metabolism is an important but not the only factor in the genesis of hepatic coma. In this review mechanisms controlling the concentration of ammonia in the blood other than disturbed liver function have been discussed. The key function of ammonia in the pyridine nucleotide cycle has been outlined and it is suggested that the function of this cycle in patients with liver disease would repay further study.

Many factors are important in the genesis of the neuropsychiatric disturbances of liver disease but disturbed ammonia metabolism plays a key role. In 1893 Hahn and colleagues observed that Eck fistula dogs had a high blood ammonia during periods of meat intoxication. The significance of this observation to clinical medicine, and studies made in the next 20 years, went unnoticed until Van Caulaert and Deviller (1932) showed that patients with liver disease had a high blood ammonia which increased when ammonium salts were given by mouth. Not only was ammonia poorly metabolised but neuropsychiatric changes were induced in many of their patients. Kirk (1936) stressed the importance of a portal collateral circulation in allowing ammonia formed in the gut to bypass the liver and cause a raised systemic blood ammonia concentration, but he did not observe any concomitant clinical change, while Gaustad (1949) described disorders of consciousness in patients with liver disease precipitated by gastrointestinal haemorrhage, oral urea, ammonium chloride, or a high protein diet. Sadly, the importance of these observations was not appreciated and made no impact on clinical practice. Indeed, misplaced extrapolation of animal experiments dictated clinical practice and, because relative protein deficiency was a cause of hepatic necrosis in animals, patients with a failing liver from any cause were given a high protein diet—if necessary by tube when they were unconscious.

The recognition and importance of the syndrome of hepatic encephalopathy only became established following the careful clinical descriptions of Adams and Foley (1949). This was soon followed by the observations of Phillips *et al.* (1952), McDermott and Adams (1954), Sherlock *et al.* (1954), White *et al.* (1955), and Summerskill *et al.* (1956), who delineated the clinical features of this disorder and

its relation to disturbed protein and ammonia metabolism. This heralded the dawn of a new era in the rational treatment of hepatic coma.

Since then Professor Sheila Sherlock has continued to contribute to our understanding of this perplexing disorder; it therefore seems appropriate to discuss some of the extrahepatic factors known to control the concentration of ammonia in the blood.

Gut

The gut was acknowledged to be the major source of ammonia following on the work of Hahn *et al.* (1893). Folin and Denis (1912) confirmed in cats that the portal venous blood ammonia rose after a meal and found that much of this was of caecal origin, for the caecal vein had the highest concentration of ammonia, which increased rapidly when the cat became constipated and fell when it was given an enema. These observations, combined with clinical evidence that a high protein diet in susceptible patients with liver disease causes a rise in blood ammonia, suggested that unabsorbed protein was deaminated by bacteria in the caecum, from where ammonia was absorbed, and also accounted for the fall in blood ammonia in a patient given a low protein diet, cathartics, and oral antibiotics. An alternative substrate was known to be urea, for blood ammonia rose in cirrhotic patients who were uraemic (Webster and Gabuzda, 1959) and when urea was given by mouth (Van Caulaert *et al.*, 1932; Gaustad, 1949; Phillips *et al.*, 1952). Urea was thought to be the main substrate because protein absorption was assumed to be extremely efficient (Borgström *et al.*, 1957; Matthews, 1971) and so changes in dietary intake were not expected to alter the amount of protein entering the caecum. It was also assumed that the gut was freely permeable

to urea and that changes in dietary protein caused a change in blood urea (Addis and Watanabe, 1917) which could diffuse into the bowel lumen and so be metabolised by bacterial urease (Summerskill, 1966).

Probably both mechanisms play a role and both assumptions are wrong. Recent studies of nitrogen balance in patients with ileostomy suggest that dietary protein is not completely absorbed by the small gut (Gibson *et al.*, 1976b). When dietary protein was increased from 40 to 100 g the ileostomy nitrogen significantly increased from 1.8 to 2.6 g per 24 hours; there was no comparable increase in faecal nitrogen from patients with an intact colon, confirming the previous observations of McCance and Widdowson (1947). Their patients' faecal nitrogen ranged from 0.7-2.19 per 24 hours, showing that the colon degrades and absorbs at least 0.5-1 g nitrogen per day. If all this were converted to ammonia it would easily account for the ammonia which enters the portal circulation. The time course of the rise in blood ammonia after a high protein meal also favours protein as a substrate. Furthermore, the whole of the gastrointestinal tract is not permeable to urea (Fordtran *et al.*, 1965), free diffusion only occurring in the duodenum and jejunum. Diffusion of urea decreases in the ileum and is virtually absent in the colon (Billich and Levitan, 1969; Bown *et al.*, 1975). Thus, most of the urea metabolised in the caecum will have diffused into the upper small bowel and then passed through the ileocaecal valve into the colon. An alternative explanation (Wolpert *et al.*, 1970) is that there is a juxtamucosal colonic urease deep to the diffusion barrier in the mucosa for urea, and that the tissue fluid urea may be metabolised, the ammonia then entering both blood stream and bowel without the urea appearing in the lumen of the colon. This attractive hypothesis was based on data from perfusion of the colon *in situ* in man when it was observed that intravenous urea was followed by an increased ammonia output from the colon. When colons from patients who had undergone the operation of colonic exclusion were studied, however, this did not occur (Bown *et al.*, 1975). The discrepancy may be due to some ileal contamination of the colon occurring in the former observations. Approximately 30% of the body urea is broken down in the gut each day (Jones *et al.*, 1969; Walser and Dlabl, 1974; Gibson *et al.*, 1976a).

The absorption of ammonia from the colon depends on non-ionic diffusion (Castell and Moore, 1971; Bown *et al.*, 1975), and for this reason it was assumed that the unabsorbable disaccharide lactulose (Bircher *et al.*, 1966), which is metabolised in the colon to volatile fatty acids, caused a fall in

blood ammonia by decreasing the colonic pH and so blocking ammonia absorption. Although faecal pH falls only slightly after oral lactulose (Zeegan *et al.*, 1970), it was shown using a telemetering pill that the pH of the caecum fell well within the range which was known to block ammonia absorption (Bown *et al.*, 1974). If this were truly how blood ammonia is reduced the faecal ammonia should rise. Using the faecal dialysis technique (Wrong *et al.*, 1965), however, no such rise in faecal ammonia was observed (Zeegan *et al.*, 1970; Agostini *et al.*, 1972). It thus seemed possible that ammonia production was inhibited by the change in pH; subsequent observations *in vitro* showed that mixed cultures of colonic bacteria continued to grow but produced less ammonia either from protein or urea at low pH (Vince *et al.*, 1973).

The ultimate application to treatment of the role of the colon in the genesis of ammonia is the use of colectomy or colonic exclusion in chronic encephalopathy. Usually patients who are not controlled by medical measures are too ill to benefit from the operation. In a control trial taking all comers, the results are unimpressive (Resnick *et al.*, 1968), but in the individual case this can be brilliant (Walker *et al.*, 1965; Gibson, 1977). Presumably the main effect of this operation is to eliminate stasis in the lower bowel so that colonic bacteria proliferating in the ileum have less time to form ammonia and other toxic products.

Recently it has been observed that germ-free animals who have undergone a portacaval anastomosis develop meat intoxication with a commensurate rise in blood ammonia after a protein meal (Nance *et al.*, 1971). Thus methods other than bacterial degradation of protein or urea must be invoked. Windmueller and Spaeth (1974) showed that ammonia is liberated from the gut mucosa during the metabolism of, for example, glutamate, which may explain this phenomenon.

Role of kidney

In 1921 Nash and Benedict showed that ammonia in the renal vein was higher than that in an artery. The ammonia produced by the kidney from deamination of amino acids acts as a urinary buffer. The proportion of ammonia excretion in the urine is governed by non-ionic diffusion; thus, in short-term experiments, if the urine is made alkaline by parenteral bicarbonate the renal vein ammonia increases, whereas an acid urine causes a fall in renal vein ammonia (Poppell *et al.*, 1956). However, this observation is of no therapeutic use because chronic acidosis increases ammonia production by the kidney (Welbourne, 1975).

Role of muscle

In 1928 it was found that ammonia could be released from muscle undergoing tetanic spasm *in vitro* and this was assumed to be due to the degradation of AMP to IMP (Embden and Wassermeyer, 1928) and, more recently, ammonia has been shown to be liberated by exercising muscle *in vitro* (Allen and Conn, 1960; Sinniah *et al.*, 1970). In the last few years observations suggest that the muscle may play a much more important role than hitherto realised in ammonia homeostasis (Lowenstein, 1972). As Daniel *et al.* (1977) pointed out, muscle mass is 10 times that of the liver and therefore may be extremely important in protein homeostasis. Patients with chronic hepatic encephalopathy on admission to hospital have tended to show spontaneous improvement, possibly due to rest in bed. For this reason, a group of patients with chronic encephalopathy were asked to climb two flights of stairs in the outpatient department. Their raised arterial ammonia level further increased after this mild exercise (Fig. 1).

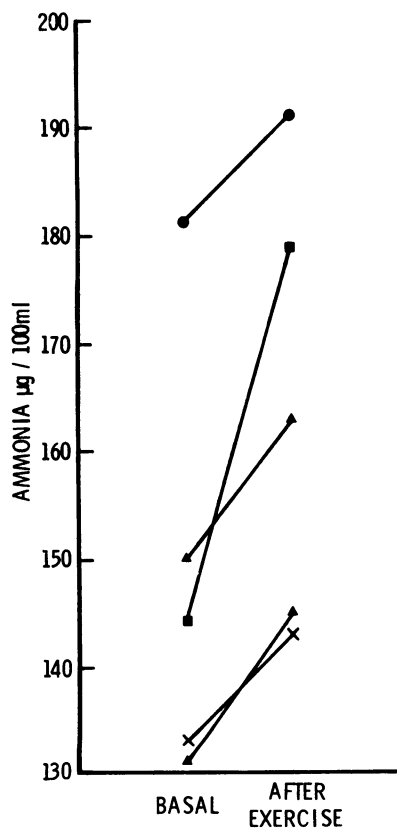


Fig. 1 Effect of mild exercise (walking up two flights of stairs) on the blood ammonia of patients with liver disease.

No change in normal blood ammonia was observed in a control group. This rise might be caused by impaired clearance of the ammonia produced by exercise, not only by the damaged liver but also by muscle. Normally peripheral tissues utilise ammonia (Bessman and Bessman, 1955) and this may be impaired in hepatic encephalopathy. Indeed, in some patients ammonia is apparently produced by such tissues (Summerskill *et al.*, 1957). On the other hand, muscles of patients with chronic encephalopathy might produce more ammonia for a given amount of work (Allen and Conn, 1960). For this reason, the production and clearance of ammonia using an isolated forearm technique was studied. By studying the arterio-deep vein difference across the forearm and simultaneously measuring blood flow by plesythmography it was shown that patients with an already high blood ammonia cleared exogenous ammonia per unit weight of tissue normally (Fig. 2). Furthermore, Fig. 3 shows that when a standard amount of work was performed by the forearm using an ergometer the actual ammonia production by patients with hepatic encephalopathy and controls did not differ. It is difficult to compare these results with those of Allen and Conn (1960) who relied on A-V difference and did not correct for changes in blood flow. It would thus seem that production and clearance of ammonia by muscle in liver disease is normal and that the rise following even gentle exertion is due to the inability of the liver to metabolise ammonia, which is released into the circulation at a normal rate. As blood ammonia does not rise on gentle exercise in normal man, the liver must be extremely efficient at clearing ammonia from the blood. Figure 4, however, confirms a little known observation that, under conditions of extreme exertion, peripheral ammonia can rise (Schwartz *et al.*, 1958), presumably because of a combination of production of an enormous ammonia load and the decrease in liver blood flow which occurs on severe exercise. The observation on the forearm raises other points. It would seem that some of the old observations of a decreased AV difference and even production of ammonia by peripheral tissues could well be an artefact of technique, for patients are often asked to flex their forearm muscles to facilitate venepuncture, and also increased basal muscle activity occurs in patients with hepatic encephalopathy.

The recent description of the purine nucleotide cycle (Lowenstein, 1972) highlights the importance of ammonia as a controlling metabolite and explains how free ammonia is derived from amino acids, which can then act as a source of energy. It also regulates the concentration of citric acid cycle intermediates and the relative concentrations of

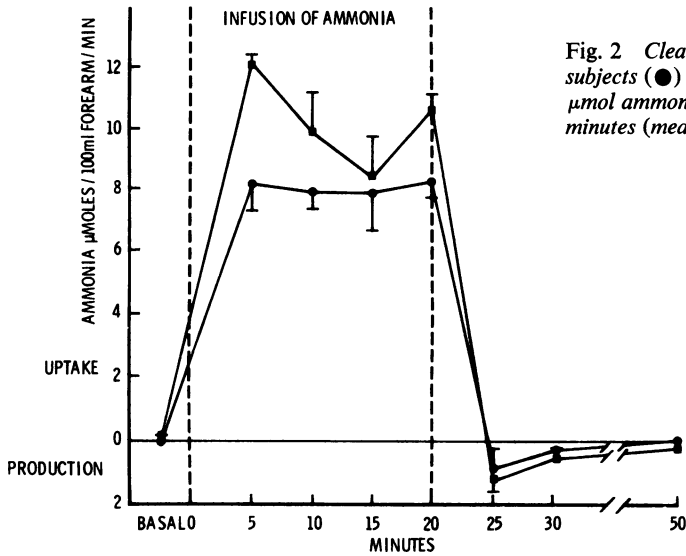


Fig. 2 Clearance of ammonia by forearm in control subjects (●) and patients with liver disease (■) (2000 μmol ammonia infused as ammonium acetate over 20 minutes (mean ± SE)).

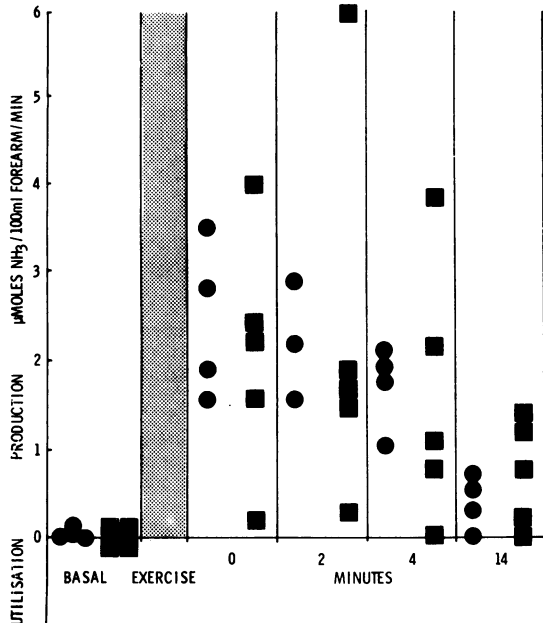


Fig. 3 Effect of exercise (10 kg/m/min for five minutes) on ammonia production by forearm in control subjects and patients with liver disease and grade 1 hepatic encephalopathy. ● Control subjects. ■ Patients with liver disease.

AMP, ADP, and ATP and the regeneration of IMP and aids the control of phosphofructokinase activity and hence glycolysis. It may be that studying the metabolism of this cycle and its possible derangements in patients with hepatic encephalopathy will give a clue as to why ammonia can be an important factor in the genesis of their symptoms.

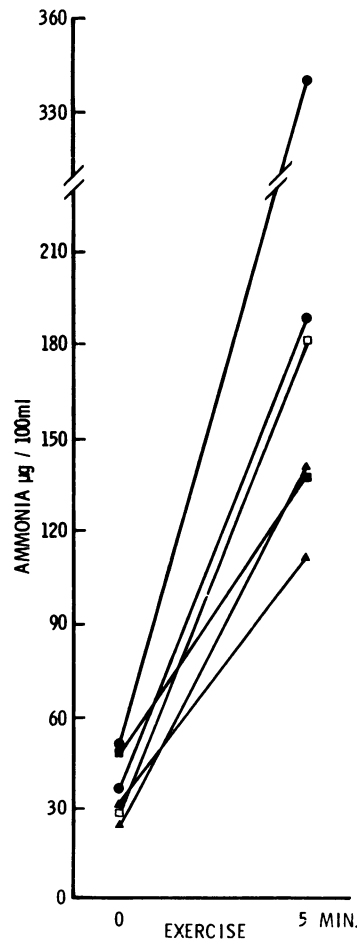


Fig. 4 Effect of severe exercise (1500 kg/m/min for five minutes) on blood ammonia in normal subjects.

I would like to thank Dr R. L. Bown for allowing me to publish his data on muscle activity and ammonia.

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