Pathogenesis of Hepatic Encephalopathy*

Z. RENO VLAHCEVIC

Veterans Administration Hospital, Richmond, Virginia 23219

Severe parenchymatous diseases of the liver, both acute and chronic, are frequently associated with hepatic encephalopathy. This term is preferable to that of hepatic coma, as it encompasses the whole spectrum of changes from bizarre alterations of behavior to various degrees of disturbance of consciousness, as well as protean neurologic manifestations. In many instances it is a reversible phenomenon, occurring either spontaneously or as the result of various therapeutic agents. The puzzling feature of hepatic encephalopathy is the discrepancy between dramatic clinical features and paucity of histopathological changes in the brain. The only histological changes encountered with regularity in patients with this entity are diffusely swollen and enlarged astrocytes (Adams and Foley, 1953). With no macroscopic or microscopic changes to account for cerebral dysfunction, it is likely that hepatic encephalopathy is caused by profound, yet undefined metabolic abnormalities.

A relationship between ammonia toxicity and cerebral dysfunction has been suspected since the discovery of "meat intoxication" in Eck fistula dogs. The clinical relationship between liver disease, cerebral dysfunction and disordered ammonia metabolism was established by Gabuzda, Phillips, and Davidson (1952) and confirmed by others.

Sources of Ammonia

The major sources of ammonia in humans are the bowel and the kidney. Ammonia is produced in the bowel by the action of bacterial and digestive enzymes on protein and urea, the major contributor probably being the right colon (Gryska and Barsamian, 1958; Silen et al., 1955; Webster, Davidson, and Gabuzda, 1958). In patients with cirrhosis, the upper small bowel, normally sterile, is contaminated by bacteria, thus contributing significantly to ammonia production (Martini et al., 1957). The reason for abnormal bacterial growth in the upper small bowel in patients with cirrhosis is not known.

Hydrolysis of the urea is an additional source of ammonia. About 25% of the endogenous urea is not excreted by the kidney but is hydrolyzed in the gastrointestinal tract at a constant rate of 0.3 mg per hour to a total of 7.2 gm of urea daily. This is equivalent in nitrogen content to 11 gm of NH_4Cl or 18 gm of protein (Walser and Bodenlos, 1959). As patients with advanced cirrhosis in terminal stages frequently develop azotemia secondary to renal failure, it is easy to visualize how hydrolysis of this excess urea in the gut could precipitate hepatic coma. All these factors, when coupled with a larger protein load, either secondary to bleeding or high protein diet, can contribute significantly to the occurrence of hepatic encephalopathy.

The second major source of ammonia is the kidney. Ammonia is produced in the kidney by deami-

* Presented at the Thirty-Ninth Annual McGuire Lecture Series, November 9-10, 1967, Medical College of Virginia, Richmond.

MCV QUARTERLY 4(1): 32-37, 1968

nation of glutamine and other amino acids, resulting in higher renal vein ammonia. The difference between renal vein and arterial ammonia is even more pronounced in patients with cirrhosis, particularly if they are potassium deficient, on diuretic therapy, or both (Weil-Malherbe, 1950; Owen et al., 1960). The mechanism for preferential secretion of ammonia into the renal vein is not known. With acetazolamide therapy, increase in renal vein ammonia was coupled with equivalent fall in urinary ammonia and a rise in urine pH. The latter may explain the shift in partition of ammonia between urine and renal vein.

The role of hypokalemia in an increased renal production of ammonia is not understood, but it can be prevented by correction of potassium deficit. In patients with liver disease who are frequently rendered hypokalemic with injudicious use of diuretics, this mechanism may be responsible for the precipitation of hepatic encephalopathy.

Removal Mechanisms and Distribution of Ammonia

Ammonia is detoxified in the liver by forming urea through the Krebs-Henseleit cycle. As a result of the marked collateral circulation and hepatocellular disease, the removal of ammonia by the liver in patients with cirrhosis is diminished. Very rarely in cases of massive fulminating liver failure, the urea cycle does not function at all, resulting in decreased blood urea nitrogen levels.

The removal of ammonia from the blood and tissues can also be accomplished through the reaction in which α-ketoglutarate combines with ammonia to form glutamic acid (Weil-Malherbe, 1950). Through the process of transamination, α-ketoglutarate can again become available either to combine with more ammonia or to continue in the tricarboxylic acid cycle (Fig. 1). Furthermore, glutamic acid can react with ammonia to form glutamine in the reaction requiring ATP.

These reactions, thus, probably serve as a defense against accumulation of ammonia, and at the same time offer a reasonable explanation for frequent elevation of glutamic acid and glutamine in patients with chronic liver disease.

Exhalation of ammonia via the lungs is a minor mechanism of ammonia removal.

As a consequence of hepatic parenchymal disease and portal-systemic shunts, the nitrogenous compounds from the portal blood bypass the liver and reach systemic circulation, resulting in the elevation of blood ammonia. Blood ammonia, however, is not a good index of "ammonia toxicity" for several reasons. First, blood ammonia correlates poorly with the state of consciousness (Moore, Strohmeyer, and Chalmers, 1963). Secondly, in the congenital defects of urea biosynthesis, blood ammonia levels may be much higher than those found in patients with hepatic coma with no neuropsychiatric manifestations (Russell et al., 1962). Finally, the experiments by Warren et al. (1960; Warren, 1958; Stabenau, Warren and Rall, 1959) have shown that another variable, pH, might play an important role in the pathogenesis of hepatic coma. They observed that the differential toxicity of various ammonium salts depended upon the change in the pH of the blood induced by a given salt.

Ammonia, in the blood or any biological fluid, exists in two forms: ammonium ion (NH_4^+) and free ammonia (NH_3^0). According to the Henderson-Hasselbalch equation—

$$pH = pK_a + \log \frac{NH_3^0}{NH_4^+}$$

the relative proportion of each component is directly dependent upon pH. Knowing the pK_a of ammonia (about 9.15), total blood ammonia (obtained by any standard microdiffusion test) and pH, one can calculate the relative proportion of ionized (NH_4^+) and un-ionized free ammonia (NH_3^0). The relative proportions of un-ionized ammonia at pH's of 6, 7, 8, and 9.15 are approximately 0.1%, 1%, 10%, and 50%. According to present concepts, only un-ionized free ammonia is capable of crossing the cell membrane. At the normal blood pH only a small proportion of ammonia is in this form. The change of pH to the alkaline side will increase the proportion of un-ionized ammonia (NH_3) and, thus, theoretically, increase the chance of inducing hepatic encephalopathy.

According to the pH gradient distribution hypothesis, distribution of ammonia from blood to tissue seems to be dependent upon a pH difference between the two compartments. If a membrane, such as

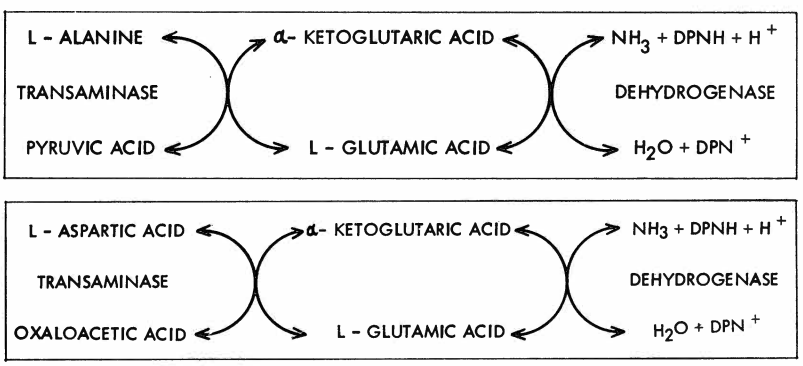

Fig. 1—Ammonia detoxication reaction involving α-ketoglutaric Acid. The transamination reactions involving glutamic acid are also presented.

the blood-cerebrospinal fluid barrier, is permeable only to un-ionized ammonia, the concentration gradient which normally exists between extra- and intracellular fluid should be maintained, depending upon the pH in each compartment. With the change of this gradient, the redistribution of un-ionized ammonia occurs, providing the change takes place on one side of the membrane only or both sides in the opposite direction. Consequently, an increase in extracellular pH, such as in metabolic alkalosis, will both increase the relative proportion of un-ionized ammonia and facilitate its transfer across the membrane, resulting in a rise of intracellular ammonia.

Respiratory and metabolic alkalosis are frequently encountered in patients with cirrhosis. Respiratory alkalosis with hyperventilation is the most frequent acid-base disturbance in these patients. The underlying mechanism for its occurrence is not clear. This type of alkalosis, however, does not cause significant change in the pH gradient, as carbon dioxide diffuses freely across the cell membranes and, consequently, no change in ammonia distribution occurs. Conversely, in patients with metabolic alkalosis, the pH gradient will be increased, as the pH change will occur on one side of the membrane only, thus resulting in redistribution of ammonia. Hypokalemic, hypochloremic alkalosis accompanied by intracellular acidosis frequently occurs in patients with cirrhosis who are treated with potent diuretics. As a result of metabolic alkalosis, formation of un-ionized ammonia (NH_3^0) is favored, and its transport across the membrane is facilitated. This mechanism may be partially responsible for the frequent precipitation of hepatic encephalopathy in cirrhotics on this therapy.

Whether aforementioned theoretical considerations play an important role in the pathogenesis of hepatic coma is not as yet entirely clear, largely because no direct method for measuring un-ionized ammonia (NH_3^0) is available.

Clinical trial with the intention of implementing these theoretical considerations in patients with hepatic coma was not successful (Warren et al., 1960). The administration of 0.1N HCL intravenously for a brief period of time, in an attempt to lower the pH, did not lead to any improvement in the state of consciousness. The duration of this clinical trial was very short and, therefore, inadequate to fully test this hypothesis.

Pathogenetic Mechanisms

Hepatic encephalopathy can be classified either as spontaneous (endogenous) or induced (exogenous). As far as the pathogenesis is concerned, this classification is somewhat arbitrary. It only implies that exogenous coma is precipitated by a variety of factors (Table 1) and, consequently, does not necessarily imply extremely poor liver function. Conversely, spontaneous or endogenous coma usually implies active, sometimes fulminating liver disease, occurring without presence of precipitating factors and, consequently, carries a worse prognosis.

The pathogenetic mechanisms involved in hepatic encephalopathy are not known. It is generally accepted, but not proven, that either ammonia or a nitrogenous substance liberating ammonia is in some way associated with the syndrome of hepatic encephalopathy. In addition to, or because of disordered ammonia metabolism, patients with hepatic encephalopathy have a markedly altered metabolism of glucose and oxygen. This is manifested by the accumulation of various intermediates of tricarboxylic acid (TCA) cycle, pyruvic and lactic acid in the blood and in the spinal fluid. Furthermore, brain oxygen uptake and cerebral blood flow in these patients are decreased between 25% and 50%. All of these facts indicate that profound metabolic biochemical changes take place in these patients, some of them affecting cerebral energy metabolism. Such disturbance of cerebral energy metabolism is now thought to be the basis of neurological changes in ammonia intoxication. Neurologic findings of hepatic encephalopathy such as asterixis, decerebrate rigidity, hyperpnea and coma suggest malfunction of the specific structures in the base of the brain and possibly their cortical connections (Magoun, 1963; Plum and Posner, 1966).

The understanding of biochemical disorders involved in hepatic coma was hampered by the impossibility of studying coma in humans. Most of the available data

TABLE 1

Precipitating Factors

Gastrointestinal Bleeding
Excessive Amounts of Nitrogenous Substances (Dietary Protein, Ammonium Salts, Urea, Amino Acids)
Azotemia
Diuretics
Metabolic Alkalosis With or Without Hypokalemia
Acute Infections
Sedatives, Hypnotics, Analgesics (Chloral Hydrate, Paraldehyde, Barbiturates, Opiates)
Major Surgical Procedures
Massive Paracentesis

are from in vitro experiments or animal models. Thus, it must be stressed with great emphasis that extrapolation of these experiments to human situations should be done with reservation and due caution.

Bessman and Bessman (1955) proposed a hypothesis which attempted to link the effect of excess ammonia in the brain with the effectiveness of the TCA cycle. The common link is α-ketoglutarate, an important intermediate of the TCA cycle. They suggested that, when an excess of ammonia is presented to the brain, depletion of α-ketoglutarate takes place through its reaction with ammonia to form glutamic acid. This reaction (Fig. 2) is catalyzed by glutamic dehydrogenase and uses DPNH, as an electron donor. Diversion of α-ketoglutarate from the TCA cycle diminishes the rate of formation of the succeeding members of the cycle. It is apparent that this will interfere with oxygen utilization and reconstitution (or formation) of adenosine triphosphate (ATP), an overall process known as oxidative phosphorylation. The formation of ATP will be diminished in direct proportion to the depletion of α-ketoglutarate.

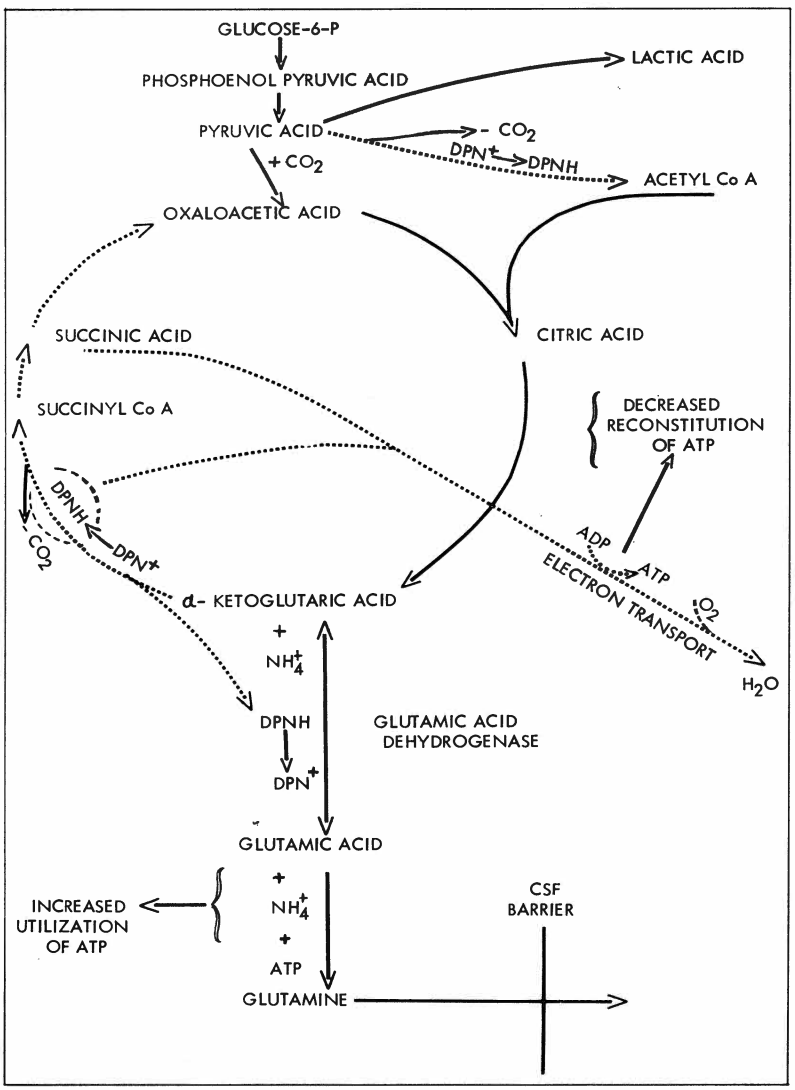

Fig. 2—Metabolic interrelationship between ammonia metabolism and TCA cycle in the brain. The dotted (. . . . .) line illustrates metabolic derangements of the TCA cycle postulated to occur as a result of excess ammonia. (Adapted with permission from *Gastroenterology* 49:699, 1965.)

The second line of defense in the process of ammonia detoxication in the brain is offered by the reaction in which glutamic acid reacts with ammonia to form glutamine (Fig. 2). This reaction requires ATP for energy and is catalyzed by glutamine synthetase. As a result of the accentuated formation of glutamine, this compound is elevated in the cerebrospinal fluid and blood in patients with hepatic coma.

Worcel and Erecinska (1962) have also shown that the addition of ammonia to rat liver mitochondria inhibited oxygen uptake when α-ketoglutarate and pyruvic acid were used as substrates. They postulated that the inhibitory action of ammonia on the respiration of rat liver mitochondria is due to competition for DPNH between glutamic dehydrogenase and electron chain transport (Fig. 2).

If these theoretical considerations are correct, when an increased amount of ammonia crosses the blood-brain barrier and reacts with α-ketoglutarate and glutamic acid, brain ATP levels should be decreased as a result of the combination of inadequate formation and excessive utilization. For the same reasons, α-ketoglutarate would be expected to be low. Schenker et al. (1967; Shorey, McCandless, and Schenker, 1967) measured ATP and α-ketoglutarate in the brain of rats who were given toxic doses of ammonium acetate. ATP and α-ketoglutarate were measured in the rapidly frozen base and cortex of the brain. Their results indicate that the ATP levels were unchanged in the cortex but definitely decreased at the base of the

brain, with ammonia concentration equal in both parts. These experiments offer for the first time in vivo evidence of the toxic effect of ammonia on energy metabolism in the brain. At the same time, a measurable decrease of phosphocreatine at the base was observed. Glucose and glycogen decreased significantly in both base and cortex.

The mechanism for this selective depletion of ATP at the base of the brain is not known, but it could be due to the impaired formation of ATP, its increased utilization, or the combination of both. The significance of selective ATP and phosphocreatine depletion at the base of the brain is not known. However, the available evidence suggests that one-third depletion of ATP is deleterious in anoxic rats (Dahl and Balfour, 1964). The role of ATP in the maintenance of proper cerebral function is also not known, though it has been postulated that energy provided by ATP may be required for proper transmission of nerve impulses (Skou, 1965). The relationship between the state of consciousness and ATP depletion was not clarified in these experiments.

The determination of α-ketoglutarate did not demonstrate any depletion of this intermediate either in the cortex or at the base of the brain. It is possible, however, that the current methods cannot assess small cerebral pools in highly differentiated compartments. These results, as well as the failure of ammonia to interfere in vitro with either respiration or phosphorylation, are not compatible with Bessman's hypothesis.

McKhann and Tower (1961) showed that the addition of ammonium chloride to the brain cortical slices caused significant reduction of oxygen uptake when pyruvate or α-ketoglutarate were used as a substrate. They concluded that ammonium interferes with oxidative decarboxylation of pyruvate and α-ketoglutarate. This observation offers another possible hypothesis applicable to the human situation. Other factors such as deficient acetyl coenzyme A, metabolic inhibitors—either not detoxified in the liver or produced as a result of ammonia intoxication—and medium chain fatty acids are a few possible candidates which have not yet been adequately studied.

Though a great step forward, these experiments have not yet offered a new concept relating to the pathogenesis of hepatic encephalopathy.

The concentrated efforts to explain hepatic encephalopathy from the standpoint of disordered ammonia metabolism heretofore have not been rewarding. Geiger (1958), however, in his experiment with perfused cat brain, has opened new avenues and new approaches. He found that cat brain preparations remained viable and were able to perform normal neurological and biochemical functions for a prolonged period of time with the liver inserted into the circulation. This was not the case when the brain preparation was perfused with the red cell suspension in the Ringer solution devoid of organic substances normally present in blood. This emphasizes the importance of the metabolic interrelationship between the liver and the brain. The implication of these experiments is that normal brain function depends upon a normally functioning liver, which may supply the brain with some important metabolic precursors such as cytidin and uridin which the brain is itself incapable of producing (Webster, 1965). In patients with cirrhosis, this metabolic interrelationship might be disturbed, resulting in abnormal brain function.

A repeat of the Geiger experiments with these thoughts in mind, as well as new and systematic approaches in other areas, might offer additional insight into the pathophysiology of this fascinating and perplexing entity, which is so inadequately understood at the present time.

References

ADAMS, R. D. AND J. M. FOLEY. Neurological disorder associated with liver disease. *A. Nerv. and Ment. Dis. Proc.* 32: 198–237, 1953.

BESSMAN, S. P. AND A. N. BESSMAN. The cerebral and peripheral uptake of ammonia in liver disease with an hypothesis for the mechanism of hepatic coma. *J. Clin. Invest.* 34: 622–628, 1955.

DAHL, N. A. AND W. M. BALFOUR. Prolonged anoxic survival due to anoxia pre-exposure: brain ATP, lactate, and pyruvate. *Am. J. Physiol.* 207: 452–456, 1964.

GABUZDA, G. J., JR., G. B. PHILLIPS, AND C. S. DAVIDSON. Reversible toxic manifestations in patients with cirrhosis of the liver given cation-exchange resins. *New Eng. J. Med.* 246: 124–130, 1952.

GEIGER, A. Correlation of brain metabolism and function by use of a brain perfusion method in situ. *Physiol. Rev.* 38: 1–20, 1958.

GRYSKA, P. F. AND E. M. BARSAMIAN. The site of ammonia production and absorption in Eck fistula dogs. *Surg. Forum* 9: 99–102, 1958.

MAGOUN, H. W. *The Waking Brain.* 2nd Edit. Springfield, Illinois: Charles C. Thomas, 1963.

MARTINI, G. A., E. A. PHEAR, B. RUEBNER, AND S. SHERLOCK. The bacterial content of the small intestine in normal and cirrhotic subjects: relation to methionine toxicity. *Clin. Sci.* 16: 35–51, 1957.

MCKHANN, G. M. AND D. B. TOWER. Ammonia toxicity and cerebral oxidative metabolism. *Am. J. Physiol.* 200: 420–424, 1961.

MOORE, E. W., G. W. STROHMEYER, AND T. C. CHALMERS, with technical assistance of I. K. Rasmusen and S. J. Gordon. Distribution of ammonia across the blood-cerebrospinal fluid barrier in patients with hepatic failure. *Am. J. Med.* 35: 350–362, 1963.

OWEN, E. E., M. P. TYOR, J. F. FLANAGAN, AND J. N. BERRY. The kidney as a source of blood ammonia in patients with liver dis-

ease: the effect of acetazolamide. *J. Clin. Invest.* 39: 288–294, 1960.

PLUM, F. AND J. B. POSNER. *The Diagnosis of Stupor and Coma.* Philadelphia: F. A. Davis, 1966.

READ, A. E., J. LAIDLAW, R. M. HASLAM, AND S. SHERLOCK. Neuropsychiatric complications following chlorothiazide therapy in patients with hepatic cirrhosis: possible relation to hypokalaemia. *Clin. Sci.* 18: 409–423, 1959.

RUSSELL, A., B. LEVIN, V. G. OBERHOLZER, AND L. SINCLAIR. Hyperammonaemia. A new instance of an inborn enzymatic defect of the biosynthesis of urea. *Lancet* 2: 699–670, 1962.

SCHENKER, S., D. W. MCCANDLESS, E. BROPHY, AND M. S. LEWIS. Studies on the intracerebral toxicity of ammonia. *J. Clin. Invest.* 46: 838–848, 1967.

SHOREY, J., D. W. MCCANDLESS, AND S. SCHENKER. Cerebral alpha-ketoglutarate in ammonia intoxication. *Gastroenterology* 53: 706–711, 1967.

SILEN, W., H. A. HARPER, D. L. MAWDSLEY, AND W. L. WEIRICH. Effect of antibacterial agents on ammonia production within intestine. *Proc. Soc. Exp. Biol. Med.* 88: 138–140, 1955.

SKOU, J. C. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.* 45: 596–617, 1965.

STABENAU, J. R., K. S. WARREN, AND D. P. RALL. The role of pH gradient in the distribution of ammonia between blood and cerebrospinal fluid, brain and muscle. *J. Clin. Invest.* 38: 373–383, 1959.

WALSER, M. AND L. J. BODENLOS. Urea metabolism in man. *J. Clin. Invest.* 38: 1617–1626, 1959.

WARREN, K. S. The differential toxicity of ammonium salts. *J. Clin. Invest.* 37: 497–501, 1958.

WARREN, K. S., F. L. IBER, W. DÖLLE, AND S. SHERLOCK. Effect of alteration in blood pH on distribution of ammonia from blood to cerebrospinal fluid in patients in hepatic coma. *J. Lab. Clin. Med.* 56: 687–694, 1960.

WEBSTER, L. T. Hepatic coma—A biochemical disorder of the brain. *Gastroenterology* 49: 698–702, 1965.

WEBSTER, L. T., JR., C. S. DAVIDSON, AND G. J. GABUZDA. Effect on portal blood ammonium of administering nitrogenous substances to patients with chronic hepatic disease. *J. Lab. Clin. Med.* 52: 501–514, 1958.

WEIL-MALHERBE, H. Significance of glutamic acid for the metabolism of nervous tissue. *Phys. Rev.* 30: 549–568, 1950.

WORCEL, A. AND M. ERECINSKA. Mechanism of inhibitory action of ammonia on the respiration of rat-liver mitochondria. *Biochim. Biophys. Acta* 65: 27–33, 1962.