LIVER AND BRAIN

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NORMAL cellular metabolism is one of the factors in normal brain function. Although the brain has its own special metabolism, it communicates through its blood-brain barrier with the body as a whole. It is not at all isolated from the rest of the organism. It seems, therefore, reasonable to accept that disturbances in the homeostasis of the body may produce abnormalities in the brain cell which will be manifested as neurological and psychiatric symptoms. This does not mean that all cerebral functional disturbances will be of metabolic origin.

In this review the correlation between disturbances of liver function and the brain will be discussed.

The Existence of Cerebral Dysfunction Mediated by the Liver

In classical antiquity the influence of liver and bile upon cerebral function was recognized, although in a different way from that of the modern point of view. It is, however, very difficult to translate the classical terminology into modern medical terms because another interpretation is given to the words. Ancient texts also describe diseases which can easily be interpreted as liver coma (Bauman, 1955).

It was in the nineteenth century that more cases of cerebral disturbances produced by liver disease were described. Griffin (1833) and Bright (1836) gave very accurate clinical descriptions. Frerichs in 1877 had an almost modern view on the relation between brain and liver. It was Eppinger (1937) who, half a century later, suggested the connection between hepatitis and acute liver atrophy.

At the turn of the nineteenth century the famous work on Eck fistulæ dogs was done at the school of Pawlow in Leningrad. They were able to produce neurological disturbances after feeding these Eck fistulæ dogs large amounts of meat. Hahn, Massen, Nencki and Pawlow (1893) called this condition a 'Fleischstücken Vergiftigung' (meat intoxication). Nencki, Pawlow and Zaleski (1896) demonstrated an increase of the arterial blood ammonia during the symptoms.

The outline of the neurological symptoms produced by liver dysfunction was slowly drawn

during the first half of this century. Winterberg (1897) and Russel (1923) separated very clearly this condition from uræmic coma. At the beginning of these studies the more severe disturbances were stressed. It was only later on that increased attention was paid to the more subtle pathological changes in the course of cirrhosis. Foetor hepaticus has been known for a long time (Frerichs, 1877). Some neurological phenomena, such as intention tremor and Parkinsonian facies, were described by Stokes, Owen and Holmes (1945), as well as the flapping tremor by Adams and Foley (1949). A very detailed synthesis of neurological and psychological symptoms was described by Zillig (1947, 1948). Many authors have added details: headach (Rolleston, 1914), xanthopsia (Frerichs, 1877, Walshe, 1951), mydriasis (Cambier, 1954), an abnormality of breathing which is mostly hyperpnea (Wilcox, 1919; Greene, 1940). Others have studied the evolution of this syndrome from its beginning right until death in liver coma. An excellent review of this problem was written by Ratnoff (1957).

They all have stressed the connection between the disturbed intermediate metabolism produced by liver insufficiency and the cerebral dysfunction.

It can be ascertained that liver pathology and its metabolic consequences influence the brain metabolism and cause cerebral dysfunction. This includes minimal psychological deviations as well as very severe liver coma.

Normal Brain Metabolism and Liver Function

Before discussing the influence of a pathological liver function it is important to review our knowledge about the normal relationship between liver and brain cells.

It can be said *a priori* that the brain cells require even more than other cells, a perfect homeostasis in which the liver plays an important role. Gallagher and others (1956) have noted that brain slices *in vitro* remain active much longer when liver extracts are added to the nutrient fluid. No glucose uptake takes place in an isolated brain preparation unless the liver is incorporated in the

experimental heart-lung-brain circuit (Geiger, Magnes, Taylor and Veralli, 1954).

Brain metabolism differs greatly from that of other organs. It is absolutely dependent upon normal glucose utilization. A glucose deficiency very rapidly produces a decrease of oxidizable substances in the brain. The available glycogen is broken down in order to compensate for this diminution. But the anaerobic metabolism is of only limited importance. Survival of the adult mammalian cerebrum in an anaerobic medium is very short. But foetal and neonatal brain tolerate much longer an oxygen-free atmosphere. It has also been demonstrated that the amount of glucose taken up by brain tissue is sufficient to account for its oxygen consumption. The cerebral respiratory quotient of the cat (Courtice, 1940), the dog (Himwich and Nahum, 1929), the monkey (Schmidt, Ketty and Pennes, 1945) and man is 1.0 (Wortis, Bowman and Goldfarb, 1940). This indicates that carbohydrate is oxidized exclusively. The R.Q. falls in excised brain tissue, but again approaches unity when glucose is supplied (Loebel, 1925).

It has been shown by *in vitro* studies that glucose is the most potent substance in supporting the oxidative functions of the brain (Loebel, 1925). It is followed by other hexoses such as fructose, galactose, mannose, etc. It was also demonstrated that lactic and pyruvic acids sustain oxidation in brain. Succinic acid and α -keto-glutarate are also oxidized (Quastel and Wheatley, 1932; McGowan, 1937). The possibility of the brain oxidizing glutamate will also be discussed below. Oxidation of fat has not yet been demonstrated.

The dependence of the brain on glucose oxidation is very well demonstrated during hypoglycæmic coma. The loss of consciousness will occur at glucose levels of approximately 25 mg./100 ml. In this state the oxygen uptake decreases from its normal of 6.8 vol. % and falls to 1.8 vol. % in the deepest coma (Himwich, Hadidian, Fazekas and Hoagland, 1939).

Although pyruvate and lactate are used *in vitro* to maintain cerebral oxidation, it is not possible to arouse a patient in hypoglycæmia with the injection of either product. This is due to the relative impermeability of the blood-brain-barrier to these substances (Stone, 1938; Maddock, Hawkins and Holmes, 1939). Glutamate has been reported to be effective in restoring consciousness. This effect will be discussed in the next section.

The dependence of brain upon an adequate hepatic supply of glucose is again demonstrated in the hepatectomized animal. After hepatectomy the animal first develops hypoglycæmic coma and convulsions. Life may be prolonged by constant glucose perfusion. An electroencephalogram

showing a typical hypoglycæmic pattern in the hepatectomized animal may only be restored to normal with glucose (Mann and Magath, 1922a, b). This entirely confirms the studies made upon hypoglycæmic patients. It was this experimental work which provided the basis for one of the theories of liver coma, that this condition was indeed a hypoglycæmic coma (Fischler, 1916). However, a fall of blood sugar was never proved in this state and glucose perfusion never aroused a patient with liver coma as it arouses one from a hypoglycæmic state.

Normal cerebral metabolism depends, as will be seen, not only on a sufficient supply of glucose, but also of thiamine. Peters and Thompson (1934) and Peters and Sinclair (1934) have shown that finely minced brain tissue from pigeons suffering from avitaminosis B₁ respire at a less than normal rate when in the presence of an oxidizable substrate and that the addition of thiamine *in vitro* restores the ability of the brain to respire normally. The effect is associated with the oxidation of pyruvate.

Glutamic acid and glutamine are, along with the oxidizable factors, important metabolites in brain chemistry. Weil-Malherbe (1950) showed that both substances constitute 40 to 80% of the total cerebral amino-carboxyl nitrogen. Glutamic acid is one of the few amino-acids which occur in appreciable amounts in the brain. In brain slices it is taken up against a concentration gradient (Stern, Eggleston, Hems and Krebs, 1949).

Glutamic acid plays a very important role in the metabolism of the amino group. It may either take up ammonia or release it. Indeed, glutamic acid can be deaminated, and this occurs in the transfer of an amino group. On the other hand, when the ammonia production is high, its ω -carboxyl group will be aminated (Fig. 1).

This function seems to be of the utmost importance for the brain, not only as a normal pathway of amino-group metabolism, but also in pathological conditions, including ammonia intoxication. The relationship of liver coma and ammonia concentration in blood and brain will be stressed.

It has been demonstrated that under normal conditions there exists a continuous production of ammonia in the brain. All of it probably never becomes free ammonia because it is immediately metabolized. But, when oxygenation stops in the brain cell, the concentration of ammonia increases almost explosively: from 0.28 μ g. % to 0.47 μ g. % in one second and 30 seconds later it rises to 1.0 μ g. % (Richter and Dawson, 1948). This phenomenon was confirmed by Krebs and others (1949). Weil-Malherbe (1950) also showed that brain slices produced large amounts of ammonia, although it is not released in this form by normal

FIG. 1.—Relation of intermediate glucose and fat metabolism with urea cycle and ammonia metabolism.

tissue in the animal. Bessman and Bessman (1955) confirmed this in man by studying the ammonia concentration of jugular bulb blood. We demonstrated the same fact in liver cells (De Groote, 1959). Liver normally takes up ammonia, but liver slices in glucose medium produce it.

Many experiments have revealed the toxicity of ammonia for the brain, but according to Krebs and Cohen (1939) it is, in a normal person, immediately metabolized in the α -ketoglutarate-glutamate system. Krebs (1935) previously had shown that liver slices could synthesize glutamine from glutamic acid and ammonium salts. These reactions are ATP (adenosine triphosphate) dependent, and therefore connected with the oxidative metabolism of pyruvic acid in the citric acid cycle which replenishes the ATP stock.

The precursors of ammonia in blood, brain or liver are not very well known. Conway (1950) postulated a deamination of adenosine nucleotide. Another explanation is being sought by some in the presence of amino-oxidase in the brain. It is possible to reduce the ammonia content of the blood by the administration of amino-oxidase inhibitors (Dawson and Sherlock, 1958; Magnenat, Ott, Frei, Delaloye and Klaus, 1962). This decrease, however, has no effect on the clinical condition of the patient. It is to be noted that Magnenat and others (1962) showed an increase in blood ammonia while perfusing a large amount (1,000 mg.) of niamid.

Concurrently with this potential intracellular ammonia production a large amount reaches the organism by intestinal absorption. This amount is mostly of colonic origin and its value has been

roughly estimated to be several grammes daily (Phear and Ruebner, 1956). It is produced by the breakdown of amino-acids by bacteria (Silén, Harper, Mawdsley and Weirich, 1955; Martini, Phear, Ruebner and Sherlock, 1957). It is possible to lower the blood ammonia by decreasing the colonic flora. This can be achieved with antibiotics (Dawson, McLaren and Sherlock, 1957) and enemas.

Weil-Malherbe (1950), summing up his own experiences, suggests that the three enzymic transformations of glutamic acid occurring in the brain (deamination, transamination and amidation) are geared together in a single system and are subservient to a single purpose: the neutralization and removal of intracellular ammonia.

Glutamine has not only an important function in cerebral ammonia metabolism, but can also maintain the respiration of liver slices. It is completely oxidized in the brain (Weil-Malherbe, 1936). Tschirgi, Gerard, Jenerick, Boyarsky and Hearon (1949) also demonstrated that glutamic acid supports, under certain conditions, the function of the nerve cells. They developed a technique for the perfusion of an isolated rat spinal cord and studied the effect of different oxidizable substrates on the functional activity of the preparation. When glucose was withheld for 2 to 4 minutes the spinal cord reflexes often disappeared. This activity returned after addition, not only of glucose, but also of glutamate, glutamine, pyruvate, isocitrate and α -ketoglutarate. Various other oxidizable substrates such as lactate, acetate, etc. were not active. The authors had no explanation for this phenomenon.

On the other hand, there seems to be no correlation with clinical and animal experience. The perfusion of glutamic acid does not terminate a hypoglycæmic coma. This is due to the slow penetration of glutamic acid into the brain cells (Klein and Olson, 1947a, b). In man the blood sugar rises after the administration of glutamic acid and a hypoglycæmia can be ended. This effect is supposed to be produced by an adrenergic mechanism, resulting in the mobilization of liver glycogen (Weil-Malherbe, 1950).

Brain Metabolism and Disturbed Liver Function

There definitely exists a close relationship between liver metabolism and brain function. It must be accepted that something happens in the liver which influences brain metabolism. In general, it may be postulated that the very specific metabolism of the brain needs a finely regulated homeostasis. The knowledge of the exact causes of the disturbances will certainly promote the prevention and the treatment of hepatic coma.

FIG. 2.—Shema of possible factors which may be implicated in producing liver coma.

It may be suggested that a pathological liver may either release toxic products from degenerated cells, or that it does not sufficiently neutralize toxic substances coming from other organs or absorbed from the gut. It is also possible that a diseased liver does not furnish the necessary amount of metabolic intermediates needed by the brain. The metabolism of brain cells may then become labile and different factors, otherwise without effect, may cause further damage and disrupt the intermediate metabolism (Fig. 2).

Psychological disturbances, decrease of consciousness and coma are very non-specific reactions. It is not unreasonable that a variety of pathological conditions which interfere with cerebral metabolism should cause similar clinical syndromes.

In order to evaluate the influence of liver pathology on cerebral metabolism a study of two aspects of this phenomenon is needed. First of all the composition of blood during liver disease has to be investigated. Indeed, blood carries different substances from liver to brain and is an important factor in homeostasis. Secondly, the influence of the resulting changes on cerebral metabolism has to be determined.

Biochemistry of Liver Disease

Many workers have studied the biochemistry of liver disease. Completeness is not the aim of this review. Only facts of importance in the study of the liver-brain relationship will be mentioned.

It is well known that the so-called liver function tests are not different in liver patients with and without cerebral disturbances (Marner, 1949; Ricketts, Kirsner and Palmer, 1950; Switzer, Steigmann, de la Huerga and Schaffner, 1952; Karl, Howell, Hutchinson and Cantanzaro, 1953;

De Groote, 1959). There is, nevertheless, some parallelism between the severity of liver dysfunction and function tests on the one hand and the disturbance of liver metabolism on the other hand. But in a particular patient these tests do not change before, during or after an episode of coma.

Numerous abnormalities in blood composition which may be connected with disturbed metabolism have been demonstrated. Most of them are important for the brain.

First of all, glucose metabolism has to be considered. Glucose has been shown to be vital for normal brain function. The coma after experimental hepatectomy in animals is due to hypoglycaemia. No other organ can replenish the brain glucose. As previously stated, its perfusion can maintain for a longer period the life of the hepatectomized animal. It has, therefore, been thought that hypoglycaemia also caused human liver coma (Fischler, 1916). Although it was demonstrated that severe liver disease could decrease blood glucose (Roth, 1937), no one has succeeded in arousing a liver-coma patient with glucose perfusion as it is possible to do in cases of hypoglycaemic coma. It is now generally accepted that hypoglycaemia plays only a minor role in clinical liver coma.

Nevertheless, many biochemical lesions in glucose metabolism have been described. The damaged liver cannot metabolize glucose as fast as a normal one. The accumulation of different intermediate metabolic products has been demonstrated: pyruvic acid (Snell and Butt, 1941; Marche and Marnagh, 1946; Lotte, 1948; Amatuzio and Nesbit, 1950; Papayannopoulos and Vasilounis, 1953; Carfagno, De Horatius, Thompson and Schwartz, 1953; Calvert, Smith and Werien, 1954; Dawson and others, 1957), α -ketoglutaric

acid (Seligson, 1952; Carfagno and others, 1953; Seitz, Engelhardt-Görkel and Shaffry, 1955; Dawson and others, 1957; Strohmeyer, Martini and Klingmüller, 1957), lactic acid (Carfagno and others, 1953; Smith, Ettinger and Seligson, 1953), citric acid (De Groote, 1959). Pyruvic acid rises much higher during a glucose tolerance test in liver patients without any thiamine deficiency as compared to normal subjects (Dawson and others, 1957).

The increase of the α -keto acids is roughly proportional to the severity of the liver disturbances, but is not correlated with the increase of blood ammonia, and the administration of ammonium chloride has no constant effect (De Schepper, 1958a; De Groote, 1959). Walshe (1955) found that the administration of glutamic acid produces no rise of pyruvic and α -ketoglutaric acids.

Berenbom and White (1950) observed that radioactive glutamic acid and glutamine disappeared very rapidly from the blood circulation, and that the amide nitrogen was excreted largely as urea and only a small amount as ammonia.

All these data are very important for a better understanding of the intermediate biochemical mechanisms of liver coma. Indeed, these substances belong directly or indirectly to the oxidative citric acid cycle which is known to be of fundamental importance in the formation and transfer of energy in intermediate metabolism.

Many indications lead us to the assumption that a disordered oxidative metabolism may increase the blood concentration of intermediate metabolites. This also happens in cases without liver pathology. The increased thyroxine concentration in cases of Basedow's disease uncouples oxidation and phosphorylation, causing a rise of blood α -keto acids. Cardiac failure results in liver congestion and anoxemia with a corresponding disturbance of glucose metabolism and high blood α -keto acid levels (Yanof, 1942).

A disturbance of the oxidative metabolism of the brain has also been shown by demonstrating a decrease of the cerebral uptake of oxygen in patients with cirrhosis and in whom there is also normal blood oxygen saturation. This decrease has been demonstrated even in the absence of neurological symptoms (Weshler, Crum and Roth, 1954; Fazekas, Howard, Ehrmantraut and Alman, 1956). The minimal amount of oxygen needed for normal cerebral function is difficult to determine. It has already been shown that cerebral oxygen uptake decreases in ageing without apparent deleterious effect. According to Fazekas and others (1956), the critical level should be situated approximately at 2.2 ml. O_2 /100 mg. of brain/min., whereas the normal level should be found at 3.3

ml. In liver patients with cerebral disturbances the mean oxygen uptake was 1.7 ml., decreasing to 1.6 ml. in comatose patients. In this latter group the lowest figure of 0.9 ml. was noted.

In addition to the abnormal glucose metabolism, which is presumed to be secondary to a common cause, an important disturbance of ammonia metabolism has been established.

It is well known that a spontaneously increased blood ammonia level or the administration of ammonium salts may, in certain circumstances, produce coma and death.

This finding originated with the famous Pawlowian school, where, at the end of the nineteenth century, Eck fistulae on dogs were performed. Nencki and Zaleski (1896) developed a method for ammonia determination and found in these dogs an increased blood ammonia when they were fed with proteins. This was later confirmed by Matthews (1922). Monguio and Krause (1934) and Mann and others (1954) produced the same symptoms in Eck fistulae dogs by injecting ammonium chloride; Bornstein (1929) and Webster and Davidson (1957) did likewise with the administration of glycine; Kinsell and others (1949) and White, Phear, Summerskill and Sherlock (1955) obtained the same results with methionine, but in this case without an elevation of blood ammonia. Burchi (1926, 1927), Rowntree (1930), Van Caulaert and Deviller (1932, 1935) made the remarkable observation that the administration of ammonium salts to cirrhotic patients could produce transient drowsiness, confusion or agitation. Van Caulaert and Deviller (1932), Kirk (1936) and Field (1953) found a spontaneous elevation of blood ammonia in similar patients. These facts received more attention after the clinical experience of Gabuzda, Philips and Davidson (1952) with ammonia-loaded ion exchange resins. The administration of these substances produced the same symptoms. Philips, Schwartz, Gabuzda and Davidson (1952) demonstrated that, given such conditions, a good correlation existed between blood ammonia and the severity of the clinical state.

Sherlock, Summerskill, White and Phear (1954), Schwartz, Philips, Seegmiller, Gabuzda and Davidson (1954), White and others (1955) and Havens and Child (1955) stressed again the toxicity of a protein-rich diet. Sherlock and others (1954) named this syndrome 'portal-systemic encephalopathy'. They wanted to indicate that the ammonia absorbed from the gut into the portal system produced toxicity in the periphery, especially in the brain.

The relation of the blood ammonia level to spontaneous liver coma was re-evaluated among the different groups of cirrhotic patients with and

without neurological symptoms. This relationship has been definitely demonstrated. Whereas in a given patient the venous blood ammonia was not well correlated with his symptoms, a better relationship was obtained when considering the arterial level (Bessman and Bradley, 1955; Lieber and Lefèvre, 1958).

Summing up the biochemical disturbances which could have an influence on cerebral metabolism, the following points must be remembered:

The elevation of arterial and venous blood ammonia.

Ammoniacal compounds produce episodes of coma.

Many disturbances in oxidative metabolism exist: elevation of intermediate metabolites such as pyruvic and α -ketoglutaric acids.

Disturbance of amino-acid metabolism: more amino-acids are found in peripheral blood.

Glutamic acid and glutamine accumulate in blood, brain and cerebrospinal fluid.

It is to be noted that these metabolic deviations may become irreversible as well as the coma resulting from them.

Hypothesis on the Pathogenesis of Liver Coma

Is it possible to bring together all these diverse data and to propose a single hypothesis concerning the origin of liver coma? Such a hypothesis should probably facilitate the prevention of the coma and rationalize its treatment.

Liver coma was formerly thought to be of hypoglycæmic origin (Fischler, 1916). This has repeatedly been disproved (Mann and Magath, 1922). Hartmann (1949) found a reduced formation of sulphates and suggested a decrease of the detoxifying power of the liver as a possible cause.

Since the early experiments of the Pawlowian school (1896) and the observations of Van Caulaert and Deviller (1932, 1933) the increase of blood ammonia received much attention. Bessman and Bessman (1955, 1958) are credited with the elaboration of a hypothesis which unites many data and explains a possible sequence of events. They relate the metabolism of the increased quantity of ammonia to the decrease in the Krebs-citric-acid-cycle intermediates and the disturbance of the energy metabolism causing brain cell damage. Their hypothesis rests on our knowledge of ammonia metabolism.

Ammonia is known to be metabolized in the body along two different pathways: in the urea cycle, or in glutamic acid and glutamine production. The former is the most important in the normal person. The incorporation of ammonia in the urea cycle is so efficient that only minute amounts of free ammonia circulate in normal peripheral blood. De Groote (1958, 1959) showed that

the major part of the ammonia measured in the blood is produced by the alkalization of the blood during its determination according to Conway (1950) or Seligson and Seligson (1951). Tobe (1961) also demonstrated that the quantity of free ammonia is negligible. He showed that this amount increased in pathological conditions. It is therefore dangerous to speculate about ammonia distribution, pH, etc., as long as we do not possess a method for measuring true blood ammonia and not the ammonia which is liberated from other substances. Another factor which has to be taken into account is the anticoagulant used. We demonstrated that oxalate and citrate cause a rapid rise of ammonia after blood collection (De Groote, 1958). The addition of heparin or versene does not have this effect (De Groote, 1958; Conn, 1960).

Along with the urea formation, the glutamic acid-glutamine system has to be considered. It is active not only in the brain, but also in muscles and elsewhere in the body. Although of primary importance in cerebral metabolism, it is only of secondary importance to the economy of the organism as a whole. When this system is depressed by the administration of acetazolamide, the blood ammonia does not rise in a normal person. It also does not rise when an infusion of ammonium chloride is given in those circumstances. After its administration the ammonia level falls with the same speed (De Groote, 1959).

Glutamic acid metabolism, by its ammonia-binding function, is of the utmost importance for the brain. Although ammonia is very toxic for this organ, it is continuously produced and accumulated by brain slices, whereas it disappears immediately from the brain *in situ*. Only trace amounts of free ammonia are to be found in the brain of an animal killed by immersion in liquid air (Richter and Dawson, 1948). Although its toxicity is proved by clinical and experimental evidence. Walshe and others (1958 a, b) were not able to demonstrate any influence of ammonia on oxidative brain metabolism in their experiment. A concentration of ammonia comparable with the toxic level in a cirrhotic patient did not change any of the parameters they used. Nevertheless, they succeeded in showing that the brain of animals with chronic liver disease produced more ammonia than did those of the normal animals. When subjected to maximal stimulation they were unable to lower their rate of ammonia production, where the controls did so by approximately 50%.

Not only intracerebral ammonia production, but also the steady stream of blood ammonia, which can be very important in pathological conditions, has to be metabolized. This occurs at the expense

of α -ketoglutarate which is produced in the oxidative citric acid cycle (Bessman and Bessman, 1955).

When large amounts of ammonia are available the whole glutamic acid metabolism is driven towards glutamine. Hence α -ketoglutaric acid is used for synthesis of glutamate and withdrawn from the citric acid cycle (Krebs, 1935; Weil-Malherbe, 1936; von Euler, Adler, Gunther and Das, 1938). Bessman and Bessman (1955) suggest that the α -ketoglutarate depletion will slow down the cycle and the rebuilding of ATP. Less ATP will be available, resulting in a decrease of cellular metabolism and ammonia-binding capacity. The blood level of ammonia will rise spontaneously or after administration of proteins or ammonium salts. There will be a decrease of cerebral α -ketoglutarate (Bessman, 1958; Clark and Eiseman, 1958). Glutamine, on the other hand, will be increased in brain and cerebrospinal fluid (Whitehead, Whittaker and Prior, 1954). Brain cells cannot restore their α -ketoglutarate content by absorbing it from the blood. They are almost impermeable to this substance. They depend almost entirely upon their own production, which becomes inadequate at the moment it is most needed. Not only in brain, but also in muscle, abnormalities in α -ketoglutarate metabolism have been shown to exist, indicating a generalized disturbance in the whole body (Summerskill, Wolfe and Davidson, 1957; De Groote, Dawson, Rosenthal and Sherlock, 1959).

Against the α -ketoglutarate depletion hypothesis of Bessman it has been stated that consciousness and neurological symptoms do not always correlate with the ammonia content of venous blood (Butt, Amatuzio, Bollman, Gabuzda, Giges, Shorov and Seligson, 1953). However, this correlation is better with the arterial level. But it is evident that the disturbance of cerebral metabolism is not only due to the actual ammonia content of the blood, but is also associated with the duration of its increase. Bessman (1958) is convinced that the α -ketoglutarate supply must be halved before symptoms appear.

More facts point in the same direction. The increase of glutamine in cerebrospinal fluid in cirrhotic patients has already been mentioned. McDermott and others (1955) and Caesar (1962) also showed an increase of cerebrospinal fluid ammonia in liver coma. In normal persons this ammonia is less than four-fifths of the blood content, but rises when neurological symptoms appear (Caesar, 1962). The ammonia is metabolized at a slower rate in liver patients. The level rises higher after an ammonium chloride test (Sherlock and others, 1954; Stahl, Bockel and Imler, 1959; De Groote, 1959) or after hæmor-

rhage (McDermott, 1957; Stahl and Bockel, 1958; Stahl and others, 1959).

Various authors are concerned with the blood pH change found in liver coma. In most cases it would seem to be a respiratory alkalosis due to a stimulation of the respiratory centre by the high blood ammonia (Roberts, Vanamee, Jacques, Lawrence and Popell, 1956; Vanamee, Popell, Gliksman, Randall and Roberts, 1956; Lawrence, Jacques, Dienst, Poppell, Randell and Roberts, 1957; Warren and Nathan, 1958; Stabeneau, Warren and Rall, 1958; Warren, Iber, Dölle and Sherlock, 1960). In this situation an increase in non-ionized ammonia is produced. Ammonia in this form penetrates much more readily through the cell membrane. According to the diffusion hypothesis proposed by Milne, Scribner and Crawford (1958), non-ionized ammonia should accumulate at the less alkaline side of the semi-permeable membrane. In a liver-coma patient with respiratory alkalosis it will be on the intracellular side. This leads to the suggestion by Jacques, Poppell, Vanamee, Lawrence and Roberts (1957) that the partial pressure (P_{NH_3}) of ammonia is probably the relevant factor in producing toxicity. According to Warren (1958), the more alkaline ammonium salt is definitely the more toxic. Lawrence and others (1957) showed that a state of alkalosis was more detrimental than acidosis in terms of the general or neurological status of dogs with elevation of total ammonia. According to Warren and others (1960), during an acid infusion with a change in arterial blood pH there was always a decrease in the correlation of cerebrospinal fluid and arterial ammonia. However, no significant change occurred in the mental status of any of the patients during or after infusion of acid or base.

The pH changes and their influence upon the ratio of non-ionized ammonia and the diffusion of weak bases through semi-permeable membranes are probably not of primary importance (Bessman and others, 1961). As mentioned above, pH changes do not influence the clinical state of the patient (Warren and others, 1960). One may accept on clinical and experimental evidence that the high arterial ammonia level and its duration are of greater significance. We must stress here again that one should discuss blood ammonia in guarded terms because of the existing dosage artifacts. Our own work and the independent studies of Tobe (1961) point in that direction.

In addition to ammonia, many other substances have been accused of producing liver coma. Diuretics, such as acetazolamide and thiazide and its derivatives, have already been mentioned. They can produce coma by electrolyte disturbances (Webster and Davidson, 1956; Magid and

FIG. 3.—Possible sequence of events in liver coma.

Forsham, 1958; Sherlock, Read, Laidlaw and Haslam, 1958; Misra and Teotia, 1960). Rissel, Schnack, Stefenelli and Wewalka (1958) and De Groote (1957) demonstrated that these products also produce liver coma in sensitive patients without any disturbance of electrolytes, blood volume or pH, but with a change in blood ammonia. Nevertheless, all these disturbances themselves produce coma in cirrhotic patients with only

slightly elevated ammonia. This state is also precipitated by the administration of barbiturates, morphine and its derivatives (Case Records, Mass. Gen. Hosp., 1940). The perfusion of protein hydrolysates and amino-acids is known to be detrimental (Webster, 1956; Fahey, 1957). It appears that these substances and disturbances can only cause symptoms in so far as the brain is already affected. They represent a supplementary load to the cells (Fig. 3).

Many unknowns remain and diligent research into these questions will certainly reveal important facts which may alter the viewpoints now commonly accepted, and thus promote a better understanding of the mechanisms and treatment of hepatic coma.

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REFERENCES

- ADAMS, R. D., and FOLEY, J. M. (1949): The Neurological Changes in the More Common Types of Severe Liver Disease, *Trans. Amer. neurol. Ass.*, **74**, 217.
- AMATUZZIO, D. S., and NESBITT, S. (1950): A Study of Pyruvic Acid in the Blood and Spinal Fluid of Patients with Liver Disease with and without Hepatic Coma, *J. clin. Invest.*, **29**, 1486.
- BAUMAN, E. D. (1955): Ueber die Erkrankungen der Leber im klassischen Altertum, *Janus*, **35**, 153; *idem*, **35**, 185.
- BERENBOM, M., and WHITE, J. (1950): The Metabolism of Intravenously Administered Ammonium Glutamate and Glutamine, *J. biol. Chem.*, **182**, 5.
- BESSMAN, S. P. (1958): Ammoniogenic Coma, the Chemistry of an Endogenous Intoxication, *Proc. Fourth Internat. Cong. Biochem.*, Vienna, p. 141.
- and BRADLEY, J. E. (1955): Uptake of Ammonia by Muscle, *New Engl. J. Med.*, **253**, 1143.
- and BESSMAN, A. N. (1955): The Cerebral and Peripheral Uptake of Ammonia in Liver Disease with an Hypothesis for the Mechanism of Hepatic Coma, *J. clin. Invest.*, **34**, 622.
- , RUDO, F., and COWLEY, R. A. (1961): The Relation of Blood pH to the Movement of Ammonia into Cerebral and Peripheral Tissues during Hypothermia, *Surgery*, **50**, 478.
- BOLLMAN, J., and MANN, F. C. (1930): Studies on the Physiology of the Liver. XVIII. The Effect of Removal of the Liver on the Formation of Ammonia, *Amer. J. Physiol.*, **92**, 92.
- BUTT, H. R., AMATUZZIO, D. S., BOLLMAN, J. L., GABUZDA, G. J., GIGES, B., SBOROV, V. M., and SELIGSON, D. (1953): The Clinical and Biochemical Features of Hepatic Insufficiency, *Gastroenterology*, **25**, 471.
- BORNSTEIN, A. (1929): Ueber die Rolle von Leber und Darm bei der Desaminierung der Aminosäuren, *Biochem. Z.*, **214**, 374.
- BRIGHT, R. (1836): Observations on Jaundice, More Particularly on the Form of the Disease which Accompanies the Diffuse Inflammation of the Substance of the Liver, *Guy's Hosp. Rep.*, **1**, 626.
- BURCHI, R. (1926): Saggio della funzionalita epatica con lo studio della ammoniemia spontanea e provocata, *Folia clin. Chim. Microscop.*, **1**, 3.
- (1927): Prüfung der Leberfunktion durch Untersuchung der spontanen und provozierten Ammoniämie, *Kongr.-Zbl. ges. inn. Med.*, **47**, 80.
- CAESAR, J. (1962): Levels of Glutamine and Ammonia and the pH of Cerebrospinal Fluid and Plasma in Patients with Liver Disease, *Clin. Sci.*, **22**, 33.
- CALVERT, R. J., SMITH, E., and WERIEN, J. P. (1954): The Biochemistry and Management of Hepatic Coma: A Review and Case Report, *Gastroenterology*, **26**, 650.
- CAMBIER, J. (1954): Le coma hépatique, *Presse Méd.*, **62**, 1352.
- CARFAGNO, S. C., DE HORATIUS, R. F., THOMPSON, C. M., and SCHWARZ, H. P. (1953): Hepatic Coma, a Clinical, Laboratory and Pathological Study, *New Engl. J. Med.*, **249**, 303.
- Case Records of the Massachusetts General Hospital (1940): No. 26152, *Ibid.*, **222**, 643.
- CLARK, G. M., and EISEMAN, B. (1958): Studies in Ammonia Metabolism. IV. Biochemical Changes in Brain Tissue of Dogs during Ammonia-induced Coma, *Ibid.*, **259**, 178.
- CONN, H. (1960): Effect of Heparin on the Blood Ammonia Determination, *Ibid.*, **262**, 1103.
- CONWAY, E. J., and COOKIE, R. (1938): Blood Ammonia and the Deaminase of Adenosine and Adenylic Acid *Nature (Lond.)*, **142**, 720.
- (1950): 'Microdiffusion Analysis and Volumetric Error', 3rd edition. London: Crosby Lockwood.
- COURTICE, F. C. (1940): The Metabolism of the Brain, *J. Neurol. Psychiat.*, **3**, 306.
- DAWSON, A. M., DE GROOTE, J., ROSENTHAL, W. S., and SHERLOCK, S. (1957): Blood Pyruvic Acid and Alpha-ketoglutaric-acid levels in Liver Disease and Hepatic Coma, *Lancet*, **i**, 392.

- , McLAREN, J., and SHERLOCK, S. (1957): Neomycin in the Treatment of Hepatic Coma, *Ibid.*, **ii**, 1263.
- and SHERLOCK, S. (1958): The Effect of the Amine Oxidase Inhibitor 'Marsilid' on Ammonium Metabolism in Liver Disease, *Clin. Sci.*, **17**, 587.
- DE GROOTE, J. (1957): L'ammoniémie au cours des comas chez les icériques, *Studi e Ricerche in Epatologia, Pressiero Scientifico, Roma*, **8**, 238.
- (1958): Critical Study of the Normal and Pathologic 'Ammonia' Content of the Blood, *Proc. world Congress Gastroenterology*. Baltimore: Williams and Wilkins.
- (1959): 'Studie van de Leverinsufficiëntie, Precoma en Coma'. Brussels: Arsacia Uitgaven.
- , DAWSON, A. M., ROSENTHAL, W. S., and SHERLOCK, S. (1959): Mécanisme et importance de la métabolisation périphérique de l'ammonium, *Acta Gastro-ent. belg.*, **22**, 323.
- DE SCHEPPER, P. (1958a): Les acides alpha-cétoniques sanguins, Etude clinique expérimentale en pathologie hépatique, *Rev. franç. Etud. clin. biol.*, **3**, 211.
- (1958b): Troubles de la conscience au cours d'affections hépatiques, *Acta Gastro-ent. belg.*, **21**, 267.
- EPPINGER, H. (1937): 'Die Leberkrankheiten'. Wien: Julien Springer.
- FAHEY, J. L. (1957): Toxicity and Blood Ammonia Rise Resulting from Intravenous Amino Acid Administration in Man: The Protective Effect of L-arginine, *J. clin. Invest.*, **36**, 1647.
- FAZEKAS, J. F., HOWARD, E. T., EHRMANTRAUT, W. R., and ALMAN, R. W. (1956): Cerebral Metabolism in Hepatic Insufficiency, *Amer. J. Med.*, **21**, 843.
- FIELD, R. A. (1953): Management of Bleeding Esophageal Varices, *Amer. Practit.*, **4**, 642.
- FISHER, C., and FALON, W. (1957): Blood Ammonia Levels in Hepatic Cirrhosis: Their Control by the Oral Administration of Neomycin, *New Engl. J. Med.*, **256**, 1060.
- FISCHLER, F. (1916): 'Physiologie und Pathologie der Leber'. Berlin: Springer.
- FRERICHS, F. T. (1877): 'Traité pratique des maladies du foie'. Traduit de l'Allemand par Duménil, L., et Pellagot, Paris: Baillière et Fils.
- GABUZDA, G. J., PHILIPS, G. B., and DAVIDSON, C. S. (1952): Reversible Toxic Manifestations in Patients with Cirrhosis of the Liver Given Cation-exchange Resins, *New Engl. J. Med.*, **246**, 124.
- GALLAGHER, C. H., JUDAH, J. D., and REES, K. R. (1956): Enzyme Changes during Liver Autolysis, *J. Path. Bact.*, **72**, 247.
- GEIGER, A., MAGNES, J., TAYLOR, A. M., and VERALLI, M. (1954): Effect of Blood Constituents on Uptake of Glucose and on Metabolic Rate of the Brain in Perfusion Experiments, *Amer. J. Physiol.*, **177**, 138.
- GREENE, C. H. (1940): Progress in Internal Medicine: Liver and Biliary Tract, A Review for 1940, *Arch. intern. Med.*, **67**, 807.
- GRIFFIN, W. (1833): On What Morbid State does the Occurrence of Coma and Sudden Death in Jaundice Occur, *Dublin J. Med. Chem. Soc.*, **4**, 347.
- HAHN, M., MASSEN, O., NENCKI, M., and PAWLOW, J. (1893): Die Eck'sche Fistel zwischen der unteren Hohlvene und der Pfortader und ihre Folgen für den Organismus, *Arch. Exp. Pathol. Pharmac.*, **32**, 161.
- HARTMANN, F. (1949): Studien über die Wirkung von d-1 Methionine im Stoffwechsel der erkrankte Leber, *I. Med. Dtsch. Arch. klin. Med.*, **196**, 412; *idem*, **196**, 439.
- HAVENS, L. L., and CHILD, C. G. (1955): Recurrent Psychosis Associated with Liver Disease and Elevated Blood Ammonia, *New Engl. J. Med.*, **252**, 756.
- HIMWICH, H. E., and NAHUM, L. H. (1929): The Respiratory Quotient of the Brain, *Amer. J. Physiol.*, **90**, 389.
- , HADIDIAN, Z., FAZEKAS, J. F., and HOAGLAND, H. (1939): Cerebral Metabolism and Electrical Activity during Insulin Hypoglycemia in Man, *Ibid.*, **125**, 578.
- JACQUEZ, J. A., POPPELL, J. W., VANAMEE, P., LAWRENCE, W., and ROBERTS, K. E. (1957): Significance of Partial Pressure of Ammonia in Ammonia Toxicity, *Clin. Res. Proc.*, **5**, 20.
- KARL, M. M., HOWELL, R. A., HUTCHINSON, J. H., and CANTANZARO, F. J. (1953): Liver Coma, with Particular Reference to Management, *A.M.A. Arch. intern. Med.*, **91**, 159.
- KINSELL, L. W., HARPER, H. A., GIESE, G. K., MARGEN, S., MCGALLIC, D. P., and HESS, J. R. (1949): Studies in Methionine Metabolism. II. Fasting Plasma Methionine Levels in Normal and Hepatopathic Individuals in Response to Daily Methionine Ingestion, *J. clin. Invest.*, **28**, 1439.
- KIRK, E. (1936): Amino Acid and Ammonia Metabolism in Liver Disease, *Acta med. scand. (suppl.)*, **77**, 1.
- KLEIN, J. R., and OLSEN, N. S. (1947): Distribution of Intravenously Injected Glutamate, Lactate, Pyruvate and Succinate between Blood and Brain, *J. biol. Chem.*, **167**, 1.
- (1947): Effect of Convulsive Activity upon Concentration of Brain Glucose, Glycogen, Lactate and Phosphates, *Ibid.*, **167**, 747.
- KREBS, H. A. (1935): Metabolism of Amino Acids. IV. The Synthesis of Glutamine from Glutamic Acid and Ammonia and the Enzymatic Hydrolysis of Glutamine in Animal Tissues, *Biochem. J.*, **29**, 1951.
- and COHEN, P. P. (1939): Glutamic Acid as Hydrogen Carrier in Animal Tissues, *Nature (Lond.)*, **144**, 513.
- , EGGLESTON, L. V., and HEMS, R. (1949): Distribution of Glutamine and Glutamic Acid in Animal Tissues, *Biochem. J.*, **44**, 159.
- LAWRENCE, W., JACQUES, J. A., DIENST, S. G., POPPELL, J. W., RANDELL, H. J., and ROBERTS, K. E. (1957): Effect of Changes in Blood pH on Plasma Total Ammonia Level, *Surgery*, **42**, 50.
- , SCHWARTZ, A. E., and RANDELL, H. J. (1958): Alterations in Blood Ammonia in Dogs following Total Hepatectomy and Abdominal Evisceration, *Surg. Gynec. Obstet.*, **107**, 69.
- LIEBER, C. S., and LEFÈVRE, A. (1958): Métabolisme ammoniacal et intermédiaire au cours du coma hépatique, *Acta clin. belg.*, **13**, 328.
- LOEBEL, R. O. (1925): Beiträge zur Atmung und Glykolyse tierischer Gewebe, *Biochem. Z.*, **161**, 219.
- LOTTE, J. L. (1948): 'Recherches sur l'acide pyruvique sanguin, La pyruvicémie des hépatiques'. Paris: Thèse.
- MADDOCK, S., HAWKINS, J. E., and HOLMES, E. (1939): Inadequacy of Substances of 'Glucose Cycle' for Maintenance of Normal Cortical Potentials during Hypoglycemia Produced by Hepatectomy with Abdominal Evisceration, *Amer. J. Physiol.*, **125**, 551.

- MAGID, G. J., and FORSHAM, P. H. (1958): Clinical Studies on the Diuretic Effect of Chlorothiazide, *Metabolism*, **7**, 589.
- MAGNENAT, P., OTT, TH., FREI, J., DELALOYE, B., and KLAUS, E. (1962): Etude du niamid dans l'encéphalopathie hépatique, *Chemotherapia*, **4**, 163.
- MANN, F. C., and MAGATH, T. B. (1922): Studies on Physiology of the Liver. III. The Effect of the Removal of the Liver on the Blood Sugar Level, *Arch. intern. Med.*, **30**, 171.
- MANN, J. D., BOLLMANN, J. L., HUIZINGA, K. A., FARRAR, T., and GRINDLAG, J. H. (1954): Blood Ammonia, Experimental and Clinical Study in Abnormalities of the Liver and Portal Circulation, *Gastroenterology*, **27**, 399.
- MARCHE, J., and MARNAGH, R. (1946): L'acide pyruvique chez les hépatiques avec et sans ictère, *C.R. Soc. Biol. (Paris)*, **140**, 785.
- MARNER, J. L. (1949): Thymol Turbidity Test, Takata Ara's Test and Gros' Test in Icteric Conditions, *Acta med. scand.*, **134**, 40.
- MARTINI, G. A., PHEAR, E. A., RUEBNER, B., and SHERLOCK, S. (1957): The Bacterial Content of the Small Intestine in Normal and Cirrhotic Subjects: Relation to Methionine Toxicity, *Clin. Sci.*, **16**, 35.
- MATTHEWS, S. A. (1922): Ammonia, a Causative Factor in Meat Poisoning in Eck-fistula dogs, *Amer. J. Physiol.*, **59**, 459.
- McDERMOTT, W. V., ADAMS, R. D., and RIDDELL, A. G. (1955): Ammonia Levels in Blood and Cerebrospinal Fluid, *Proc. soc. Exper. Biol. Med.*, **88**, 380.
- (1957): A Simple Discriminatory Test for Upper Gastro-intestinal Hemorrhage, *New Engl. J. Med.*, **257**, 1161.
- MCGOWAN, G. K. (1937): Pyruvate Oxidation in Brain. II. Oxygen Pyruvate Ratio and Respiratory Quotient, *Biochem. J.*, **31**, 1627.
- MILNE, M. D., SCRIBNER, B. H., and CRAWFORD, M. A. (1958): Non-ionic Diffusion and the Excretion of Weak Acid and Bases, *Amer. J. Med.*, **24**, 709.
- MISRA, S. J., and TEOTIA, J. P. S. (1960): Hepatic Coma Precipitated by Chlorothiazide in Hepatic Cirrhosis with Ascites, *Lancet*, **i**, 464.
- MONGUIO, J., and KRAUSE, F. (1934): Ueber die Bedeutung des NH_3 Gehältes des Blutes für die Beurteilung der Leberfunktion. Studien am normalen, lebergeschädigten und Eckschen Fistel Hund, *Klin. Wschr.*, **13**, 1142.
- NENCKI, M., and ZALESKI, Z. (1896): Ueber die Bestimmung des Ammoniaks in thierischen Flüssigkeiten und Geweben, *Arch. exp. Path. Pharmac.*, **37**, 385.
- , PAWLOW, J. P., and ZALESKI, J. (1896): Ueber den Ammoniakgehalt des Blutes und der Organe und die Harnstoffbildung bei den Säugetiere, *Ibid.*, **37**, 26.
- PAPAYANNOPOULOS, G., and VASILOUNIS, K. (1953): Beitrag zur Kenntnis des Brenztrauben- und Milchsäurestoffwechsels bei gewissen pathologischen Prozessen, *Dtsch. med. Wschr.*, **78**, 840.
- PETERS, R. A., and SINCLAIR, H. M. (1934): Thiamine Insufficiency and Pyruvic Acid, *Biochem. J.*, **27**, 1910.
- and THOMPSON, R. H. S. (1934): Pyruvic Acid as an Intermediary Metabolite in the Brain Tissue of Avitaminous and Normal Pigeons, *Ibid.*, **28**, 916.
- PHEAR, E. A., and RUEBNER, B. (1956): *In vitro* Production of Ammonium and Amines by Intestinal Bacteria in Relation to Nitrogen Toxicity as a Factor in Hepatic Coma, *Brit. J. exper. Path.*, **37**, 253.
- , SHERLOCK, S., and SUMMERSKILL, W. H. J. (1955): Blood-ammonium Levels in Liver Disease and Hepatic Coma, *Lancet*, **263**, 836.
- PHILIPS, G. B., SCHWARTZ, R., GABUZA, G. J., and DAVIDSON, C. S. (1952): The Syndrome of Impending Hepatic Coma in Patients with Cirrhosis of the Liver Given Certain Nitrogenous Substances, *New Engl. J. Med.*, **247**, 239.
- QUASTEL, J. H., and WHEATLEY, A. H. M. (1932): Oxidation by the Brain, *Biochem. J.*, **26**, 725.
- RATNOFF, O. D. (1957): The Liver: Three Aspects of Hepatic Failure—Cholemia, Ascites and Hemorrhagic Phenomena, in 'Analytical Pathology', p. 210. Ed. R. C. Mellow. New York, Toronto and London: McGraw-Hill Book Co. Inc.
- RICHTER, D., and DAWSON, R. M. C. (1948): The Ammonia and Glutamic Content of the Brain, *J. biol. Chem.*, **176**, 1299.
- RICKETTS, W. E., KIRSNER, J. B., and PALMER, W. L. (1950): Biliary Cirrhosis, *Gastroenterology*, **16**, 404.
- RISSEL, E., SCHNACK, H., STEFANELLI, N., and WEWALKA, F. (1958): Auslösung von Coma Hepaticum bei Lebercirrhosen durch Carboanhydraseblocker, *Schweiz. med. Wschr.*, **88**, 794.
- ROBERTS, K. E., VANAMEE, P., JACQUES, J., LAWRENCE, W., and POPPELL, J. W. (1957): Ammonia Toxicity, *J. S.C. med. Ass.*, **53**, 1.
- ROLLESTON, H. D. (1914): 'Diseases of the Liver, Gall-bladder and Bile Ducts'. London: Macmillan.
- ROTH, O. (1937): Beobachtungen bei Coma Hepaticum, *Helv. med. Acta*, **1**, 77.
- ROWNTREE, L. G. (1930): Certain Clinical and Terminal Pictures in Hepatic Disease, *Med. Clin. N. Amer.*, **13**, 1399.
- RUSSELL, D. S. (1923): Ammonia Content of the Blood in Nephritis, *Biochem. J.*, **17**, 72.
- SCHMIDT, C. F., KETTY, S. S., and PENNES, H. H. (1945): The Gaseous Metabolism of the Brain of the Monkey, *Amer. J. Physiol.*, **143**, 33.
- SCHWARTZ, K., PHILIPS, G. B., SEEGMILLER, E., GABUZA, G. J., and DAVIDSON, C. S. (1954): Dietary Protein in the Genesis of Hepatic Coma, *New Engl. J. Med.*, **251**, 685.
- SEITZ, W., ENGELHARDT-GÖRKEL, A., and SHAFFRY, I. (1955): Über die Desaminierung der Glutaminsäure bei Leberkranken, *Klin. Wschr.*, **33**, 1046.
- SELIGSON, D., and SELIGSON, H. (1951): A Microdiffusion Method for the Determination of Nitrogen Liberated as Ammonia, *J. lab. Clin. Med.*, **38**, 324.
- , MCCORMICK, G. J., and SBOROV, V. (1952): Blood Ketoglutarate and Pyruvate in Liver Disease, *J. clin. Invest.*, **31**, 661.
- SHERLOCK, S. (1958): Pathogenesis and Management of Hepatic Coma, *Amer. J. Med.*, **24**, 805.
- , READ, A. E., LAIDLAW, J. L., and HASLAM, R. (1958): Chlorothiazide in Liver Disease, *Ann. N.Y. Acad. Sci.*, **71**, 430.
- , SUMMERSKILL, W. H. J., WHITE, L. P., and PHEAR, E. A. (1954): Portal-systemic Encephalopathy, *Lancet*, **267**, 453.
- SILEN, W., HARPER, H. A., MAWDSLEY, D. L., and WEIRICH, W. L. (1955): Effects of Antibacterial Agents on Ammonia Production within Intestine, *Proc. soc. Exp. Biol. Med.*, **88**, 138.

- SMITH, M., ETTINGER, R., and SELIGSON, D. (1953): A Comparison of the Metabolism of Fructose and Glucose in Hepatic Disease and Diabetes Mellitus, *J. clin. Invest.*, **32**, 273.
- SNELL, A. M., and BUTT, H. R. (1941): Hepatic Coma: Observations Bearing on its Nature and Treatment, *Trans. Ass. Amer. Physns*, **56**, 321.
- STABENAU, J. R., WARREN, K. S., and RALL, D. P. (1959): The Role of pH Gradient in the Distribution of Ammonia between Blood and Cerebrospinal Fluid, Brain and Muscle, *J. clin. Invest.*, **38**, 373.
- STAHL, J., ROGER, S., and BOCKEL, R. (1955): Signification de l'ammoniémie provoquée au cours de diverses affections hépatiques, *Acta gastro-ent. belg.*, **10**, 846.
- and BOCKEL, R. (1958): L'ammoniémie post-hémorragique, sa valeur dans le diagnostic des hémorragies digestives, *Rev. franç. Étud. clin. biol.*, **3**, 593.
- and IMLER, M. (1959): L'ammoniémie artérielle post-hémorragique dans le diagnostic des hémorragies digestives, *Ibid.*, **4**, 462.
- STERN, J. R., EGGLESTON, L. V., HENS, R., and KREBS, H. A. (1949): Accumulation of Glutamic Acid in Isolated Brain Tissue, *Biochem. J.*, **44**, 410.
- STOKES, J. F., OWEN, J. R., and HOLMES, E. G. (1945): Neurological Complications of Infective Hepatitis, *Brit. med. J.*, **ii**, 642.
- STONE, W. E. (1938): The Effect of Anæsthetics and of Convulsants on the Lactic Acid Content of the Brain, *Biochem. J.*, **32**, 1908.
- STROHMMEYER, G., MARTINI, G. A., and KLINGMULLER, V. (1957): Veränderungen von alpha-Ketoglutaräure, Brenztraubensäure und Diphosphopyridinnucleotid bei chronischen Leberkranken, *Klin. Wschr.*, **35**, 385.
- SUMMERSKILL, W. H. J., WOLFE, S. J., and DAVIDSON, C. S. (1957): The Metabolism of Ammonia and α -keto Acids in Liver Disease and Hepatic Coma, *J. clin. Invest.*, **36**, 361.
- SWITZER, J. L., STEIGMANN, F., DE LA HUEGA, J., and SCHAFFNER, F. (1952): Hepatic Tests in Preterminal Hepatic Insufficiency, *Amer. J. dig. Dis.*, **19**, 241.
- TOBE, B. A. (1961): The Metabolism of the Volatile Amines. I. The Ammonia Content of Blood, *Canad. med. Ass. J.*, **84**, 767.
- TRAEGER, H. S., GABUZDA, G. J., BALLOU, A. N., and DAVIDSON, C. S. (1953): Blood 'Ammonia' Concentration in Liver Disease and Liver Coma, *Metabolism*, **3**, 99.
- TSCHIRGI, R. D., GERARD, R. W., JENERICK, H., BOYARSKY, L. L., and HEARON, J. Z. (1949): *Fed. Proc.*, **8**, 166.
- VANAMEE, P., POPPELL, J. W., GLIKSMAN, A. S., RANDALL, H. T., and ROBERTS, K. E. (1956): Respiratory Alkalosis in Hepatic Coma, *Arch. intern. Med.*, **97**, 762.
- VAN CAULAERT, C., and DEVILLER, C. (1932): Ammoniémie expérimentale après ingestion de chlorure d'ammonium chez l'homme à l'état normal et pathologique, *C.R. Soc. Biol. (Paris)*, **111**, 50.
- , — and HALFF, M. (1932): Le taux de l'ammoniémie dans certaines affections hépatiques, *Ibid.*, **111**, 735.
- , — and URBAN, M. (1933): La formation de l'ammoniaque dans le sang, *Ibid.*, **112**, 293.
- VON EULER, H., ADLER, A., GUNTHER, G., and DAS, N. B. (1938): Ueber den enzymatischen Abbau und Aufbau der Glutaminsäure, *Zschr. Physiol. Chem.*, **254**, 4.
- WALSHE, J. M. (1951): Observations on the Symptomatology and Pathogenesis of Hepatic Coma, *Quart. J. Med.*, **20**, 42.
- (1955): Glutamic Acid in Hepatic Coma, *Lancet*, **ii**, 1235.
- , DE CARLI, L., and DAVIDSON, C. S. (1958a): Some Factors Influencing Cerebral Oxidation in Relation to Hepatic Coma, *Clin. Sci.*, **17**, 11.
- , — (1958b): Some Factors Influencing Cerebral Ammonia Production in Relation to Hepatic Coma, *Clin. Sci.*, **17**, 27.
- WARREN, K. S. (1958): The Differential Toxicity of Ammonium Salts, *J. clin. Invest.*, **37**, 497.
- and NATHAN, D. G. (1958): The Passage of Ammonia Across the Blood-Brain Barrier and its Relations to Blood pH, *J. clin. Invest.*, **37**, 1724.
- , IBER, F., DÖLLE, W., and SHERLOCK, S. (1960): Effect of Alterations in Blood pH on Distribution of Ammonia from Blood to Cerebrospinal Fluid in Patients in Hepatic Coma, *J. lab. Clin. Med.*, **56**, 687.
- WEBSTER, L. T. (1956): Hepatic Coma: Impending Hepatic Coma and Increased Blood Ammonium Concentrations during Protein Hydrolysate Infusions, *Clin. Res. Proc.*, **4**, 146.
- and DAVIDSON, C. S. (1956): Production of Impending Hepatic Coma by a Carbonic Anhydrase Inhibitor Diammonium, *Proc. soc. Exp. Biol. Med.*, **91**, 27.
- (1957): Impending Hepatic Coma and Increased Blood Ammonium Concentrations during Protein Hydrolysate Infusion, *J. lab. Clin. Med.*, **50**, 1.
- and GABUZDA, G. J. (1957): Effect on Portal Blood Ammonium Concentration of Administering Methionine to Patients with Hepatic Cirrhosis, *Ibid.*, **50**, 426.
- WEIL-MALHERBE, H. (1936): Metabolism of Glutamic Acid in Brain, *Biochem. J.*, **30**, 665.
- (1950): Significance of Glutamic Acid for the Metabolism of Nervous Tissue, *Physiol. Rev.*, **30**, 549.
- WESHLER, R. L., CRUM, W., and ROTH, J. L. A. (1954): The Blood Flow and Oxygen Consumption of the Human Brain in Hepatic Coma, *Clin. Res. Proc.*, **2**, 74.
- WHITE, L. P., PHEAR, E. A., SUMMERSKILL, W. H., and SHERLOCK, S. (1955): Ammonium Tolerance in Liver Disease: Observations Based on Catheterization of the Hepatic Veins, *J. clin. Invest.*, **34**, 158.
- WHITEHEAD, T. P., WHITTAKER, S. R. F., and PRIOR, A. P. (1954): Hepatic Coma, *Lancet*, **266**, 43.
- WILCOX, W. H. (1919): Jaundice: With Special References to Types Occurring During the War, Lessonian Lectures, *Lecture III, Brit. med. J.*, **i**, 671.
- WINTERBERG (1897): Ueber den Ammoniakgehalt des menschlichen Blutes unter physiologischen und pathologischen Verhältnissen, *Wien. Klin. Wschr.*, **11**, 330.
- WORTIS, H., BOWMAN, K. M., and GOLDFARB, W. (1940): Human Brain Metabolism: Normal Values and Values in Certain Clinical States, *Amer. J. Psychiat.*, **97**, 552.
- YANOF, Z. A. (1942): Blood Pyruvic Acid in Heart Disease, *Arch. intern. Med.*, **69**, 1005.
- ZILLIG, G. (1947): Neurologische und Psychische Störungen bei Lebererkrankungen, *Nervenarzt*, **18**, 297.
- (1948): Neurologische und Psychopathologische Befunde bei Lebererkrankungen, *Arch. Psychiat. Nervenkr.*, **181**, 21.