CHANGES IN THE AMMONIA AND GLUTAMIC ACID CONTENTS OF THE BRAIN OF ANIMALS FOLLOWING SURGICAL INTERFERENCE WITH LIVER FUNCTION

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The effects on central nervous activity of disturbances in liver function were demonstrated many years ago by the researches of I. P. Pavlov, I. Zalesskii and M. Nencki [10], whose findings have since been confirmed by numerous workers, including ourselves, in both clinical and experimental investigations.

It is known that liver diseases are associated with various metabolic disturbances. Very serious disturbances of ammonia metabolism are encountered in such conditions as hepatic cirrhosis, hepatic coma, and establishment of an Eck fistula. At the same time, disturbances in the activity of the central nervous system are observed, being particularly pronounced in hepatic coma.

Many workers support the view that development of the comatose condition is due chiefly to the toxic action of ammonia on the nervous system [14, 15, 19]. For this reason, the use of glutamic acid for the treatment of hepatic coma has been introduced of recent years, and we have found it useful for the prevention of poisoning by ammonia, in animal experiments [1] and for the treatment of patients suffering from liver diseases.

I. P. Pavlov and M. Nencki [10] and S. S. Salazkin [11] showed that the output of urinary ammonia of dogs with an Eck-Pavlov fistula rose after feeding with meat or ammonium citrate. It was also found that the glutamine content of the brain of dogs rose after hepatectomy [17]. A rise in the ammonia content of the brain has also been observed in prolonged narcolepsy, due to impairment of the urea-synthesizing function of the liver [9]. Determinations of differences between the ammonia contents of arterial and venous blood of patients suffering from liver disease have established that ammonia is taken up by the brain when the ammonia content of arterial blood exceeds 0.1 mg % [14].

The object of the present research was to find out whether the preformed ammonia content of the brain rises following impairment of liver function. In view of its important role in ammonia metabolism, we also determined the glutamine content of the brain.

EXPERIMENTAL METHODS

Surgical procedures were applied for infliction of injury to the liver, such as are used clinically for the treatment of liver diseases. In an earlier reseach we had damaged the liver by carbon tetrachloride poisoning.*

In order to reproduce the conditions arising after an Eck-Pavlov operation we applied the operation of constriction of the portal vein, followed by its ligation, as devised by M. V. Shepelev for cats [12]. This operation

* "Effects of disturbances of hepatic function on nitrogen metabolism," Trudy Inst. Fiziol. im. I. P. Pavlova, 1958, Vol. VIII.

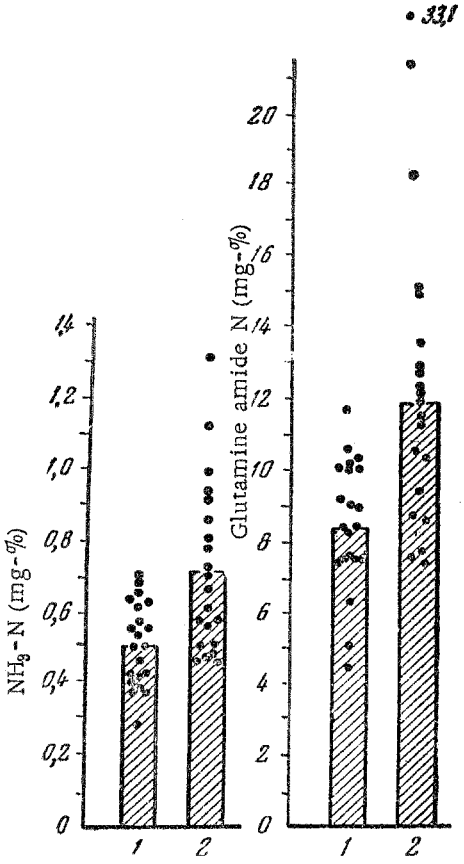

Fig. 1. Ammonia and glutamine contents of the cerebral cortex of cats before and after ligature of the portal vein.
1) Normal values; 2) after ligature of the portal vein. The value of 33.1 mg% of glutamine was not taken into account in calculating the mean value.

causes a rise in blood ammonia content [7]. In the given experiments, the portal vein was ligated 3 weeks after its preliminary constriction, and the brain of the cat was removed 6-8 days later, for ammonia and glutamine determination. The cat was rolled up firmly in a towel, and, after it had ceased to struggle, the head was plunged deeply into liquid oxygen. After it had been frozen we severed the head, extracted the frozen brain, and analyzed it for ammonia and glutamine. The analytical procedure used was that of E. A. Vladimirova [5].

EXPERIMENTAL RESULTS

The results of our experiments are shown in Figure 1. It is evident that ligature of the portal vein of cats causes an increase in the ammonia and glutamine contents of the brain (by 42 and 40%, respectively, on the average). The analytical values found in different experiments showed a very wide scatter, due probably to differences in the rate and the extent of establishment of collateral circulation after the preliminary stenosing operation. Comparison of the analytical results with those of inspection of the abdominal cavitiy during the second operation and after decapitation showed that the ammonia and glutamine contents of the brain rose when the collateral circulation was achieved chiefly by development of portocaval anastomoses, but not when it involved chiefly hepatopetal anastomoses.

In our next series of experiments we attempted to perform the operations on rats. Application of the constricting ligature to the portal vein of rats presented no difficulties, but we found that access to the portal vein for its total ligation 2-3 weeks later was very difficult, owing to formation of extensive adhesions of the omentum and the viscera to each other and to the liver. We were therefore obliged, in some operations, to restrict ourselves to a one-stage operation: the constriction of the portal vein was made as complete as possible, short of total obliteration (with the risk of causing the death of the animal, which followed in some cases), with the expectation that the gradual obliteration of the vein would follow, as is indicated by the literature [12, 20].

As in the experiments with cats, the values found for the ammonia and glutamine contents of rat brains showed a wide scatter, depending on the nature and extent of the changes in hepatic circulation. In those cases in which the autopsy showed that the portal vein was patent the ammonia and glutamine contents were within the normal range. Where the portal vein was obstructed, and where there was evidence of development of portocaval anastomoses, we found a certain increase in the ammonia and glutamine contents of the brain (Fig. 2, 3).

In a special series of experiments the rats were subjected to partial (two thirds) hepatectomy. This operation is easily performed, and is practically bloodless. The brain was removed for analysis on the fifth day after the operation, since by that time the effect of operational trauma are no longer in evidence, while only slight regeneration of hepatic tissue will have taken place [18]. Since this procedure did not give sufficiently conclusive results (see Fig. 2, 4) we increased the extent of interference with liver function by applying a constricting ligature to the portal vein, during the partial hepatectomy operation. We then found an appreciable rise in both the ammonia and the glutamine content of the brain (see Fig. 2,5).

In the next series of experiments the liver was totally excluded from the circulation, by removing the stomach and intestines, and ligating all the vessels entering or leaving the liver. The survival time after this operation was usually 5-6 hours, or somewhat more. The brain was taken for analysis 3-5 hours after the operation, and the results showed a definite elevation of the ammonia and glutamine contents (see Fig. 2, 6).

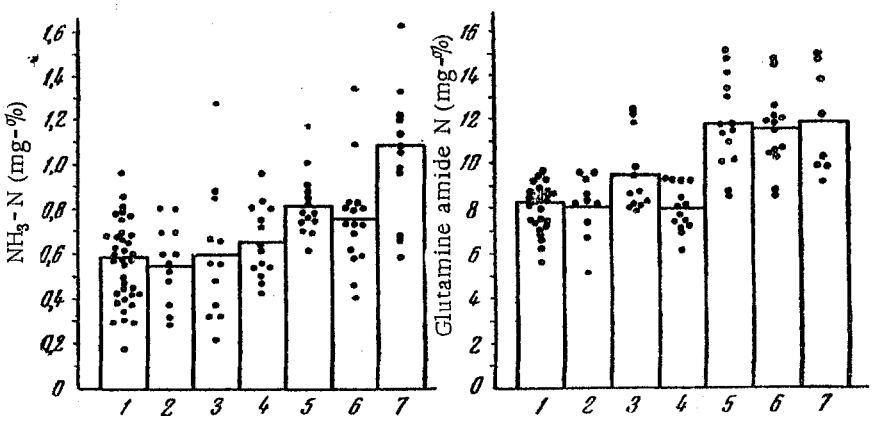

Fig. 2. Ammonia and glutamine contents of the cerebral cortex of normal and operated rates.
1) Normal; 2) control laparotomy; 3) constriction of the portal vein; 4) partial hepatectomy; 5) partial hepatectomy with constriction of the portal vein; 6) by-passing the circulation of the liver; 7) subtotal (90%) hepatectomy. Mean ammonia content – 4.18.

In the last series we performed a subtotal hepatectomy, removing 90% of the initial weight of the liver, and these experiments showed that the ammonia and glutamine contents of the brain had risen within 4-5 hours of the operation (see Fig. 2, 7).

It is well known, that apart from the ammonia entering the brain with its blood supply, ammonia may be rapidly formed in the brain, and its content may rise, following various forms of stimulation of the central nervous system; for this reason special precautions have to be taken to avoid exciting the rats when the normal brain ammonia content is to be determined [6], since otherwise a wide scatter of the results is obtained, with a high mean value for the normal ammonia content (see Fig. 2, 1).

It appears from our results that, however great might be the effects of differences in the handling and behavior of animals on the ammonia and glutamine contents of their brains, the values found for animals with liver damage were all much higher than for normal animals, even in the chronic experiments (see Figs. 1 and 2, 5). This difference may quite certainly be ascribed to the raised content of ammonia in the blood entering the brain. In the acute experiments, although the animals rapidly regained consciousness (within 5-10 minutes), and assumed their usual sitting posture, or even performed active movements, they then became progressively less active, their legs splayed out and became unable to support the weight of the body, and shortly before their death the animals lay motionless on their sides. Thus in our acute experiments the rats were in a state of depression for several hours, but regardless of this the ammonia content of the brain was elevated, due to the ammonia entering the brain with its blood supply (see Fig. 2, 6 and 7).

We observed much the same effect in our earlier experiments on dogs with an Eck-Pavlov fistula; meat feeding caused a depressive state in these animals, and was associated with a high blood-ammonia level [2]. As far back as 1894, N. O. Yurinskii [13], working under the direction of I. P. Pavlov, investigated the reasons for the stimulatory and inhibitory effects of annonia on the central nervous system, and, on the basis of his own findings and of those of other authors, he concluded that it had a dual activity. É. É. Martinson and L. Ya. Tyakhepyl'd [9], in their experiments on prolonged narcotic sleep (7-15 days), found considerably raised levels of ammonia in the brain, which could, in their opinion, cause both depression and stimulation. Incidentally, we think it significant that these authors found that animals in barbiture-induced sleep were highly resisistant to the toxic effects of large doses of ammonium chloride, as compared with control animals. We found a similar effect in dogs with an Eck-Pavlov fistula; when meat was fed after prolonged previous administration of sodium bromide the characteristic features of meat intoxication did not develop – there was no vomiting, and the dogs slept most of the time, while the blood ammonia level was raised.

What is the mechanism of the toxic action of ammonia on the brain?

Apart from the view that this is due to interference with synthesis and breakdown of acetylocholine, there is a different approach to the elucidation of the mechanism whereby ammonia exerts its toxic effect. Various authors have ascribed the neurologic changes in the central nervous system to interference with the normal functioning of oxidative processes in the brain. According to these authors, ammonia present in the blood entering the brain enters into combination not only with glutamic acid, forming glutamine, but also with α-oxoglutaric acid, which is an important substrate of the tricarboxylic acid cycle. By combining with large amounts of α-oxoglutaric acid (to form glutamic acid, and, from this, glutamine), ammonia causes a lowering of the amounts of the subsequent stages of the cycle, and hence to a lowered oxygen uptake, and this interferes with the oxidative metabolism of the brain [8, 14, 15, 19]. When glutamine is formed exclusively from preformed glutamic acid these authors consider that the oxygen uptake is raised, and that the free glutamic acid reserves of the brain are gradually used up. This view is supported by the findings of S. Bessman and A. Bessman [14] and of I. Fazekas, H. Ticktin, W. Ehrmantraut et al. [16] that the oxygen uptake of the brain in hepatic insufficiency and hepatic coma is in the fact lowered, and of E. Flock [17] that there is a large increase in the glutamine content of the brain of hepatectomized dogs, while the glutamic acid content remains unchanged.

Our results also show a considerable increase in brain glutamine content due either to carbon tetrachloride poisoning or to surgical intervention, such as ligature of the portal vein (see Fig. 1), partial hepatectomy coupled with constriction of the portal vein, by-passing the liver from the circulation, and subtotal hepatectomy (90% extirpation) (see Fig. 2, 5-7).

The distrubances in central nervous activity observed in patients suffering from hepatic insufficiency may thus be ascribable, to a certain extent, to the toxic effect of the raised content of ammonia in the brain. For this reason the treatment of such condition should be such as to promote the elimination of the excess ammonia from the organism.

SUMMARY

The ammonia and glutamine contents of the brain of cats and rats were found to be raised following surgically induced hepatic insufficiency (by constriction and ligature of the portal vein, by exclusion of the liver from the circulation, and by partial and subtotal hepatectomy). The effects of ammonia on the central nervous system are related not only to its combination with glutamic acid, but also to its reaction with α-oxoglutarate; removal of this tricarboxylic acid cycle metabolite may interfere with the normal oxidative metabolism of brain tissue.

LITERATURE CITED

[1] N. V. Veselkin and B. G. Gordon, Doklady Akad. Nauk SSSR 107, No. 2, 333-336 (1956).

[2] N. V. Veselkin and B. G. Gordon, Trudy Inst. Fiziol. im. I. P. Pavlova, 5, 425-439 (1956).

[3] N. V. Veselkin and B. G. Gordon, Byull. Éksper. Biol. i Med. 39, No. 5, 43-45 (1955).

[4] N. V. Veselkin and B. G. Gordon, Voprosy Med. Khim. 1, No. 6, 437-443 (1955).

[5] E. A. Vladimirova, Byull. Éksper. Biol. i Med. 29, No. 3, 219-221 (1950).

[6] E. A. Vladimirova, Doklady Akad. Nauk SSSR 106, No. 5, 937-940 (1956).*

[7] B. G. Gordon and M. V. Shepelev, Byull. Éksper. Biol. i Med. 42, No. 11, 23-28 (1956).*

[8] M. F. Gulyi, L. A. Mikhailovskaya and R. G. Degtyar', Proc. 8th All-Union Congress of Physical., Biochem. and Pharmacol. pp. 194-195 (Moscow, 1955);* * Proc. 1st Sci. Meeting on Problems of Fats in Nutrition, p. 29 (Leningrad, 1958).

[9] É. É. Martinson and L. Ya. Tyakhepyl'd, Problems of the Biochemistry of the Nervous System, pp. 176-188 (Kiev, 1957).* *

[10] I. P. Pavlov (with I. Zalesskii and M. Nencki) Complete Collected Works, Vol. 2, Book 1, 287 (Moscow-Leningrad, 1951).* *

* Original Russian pagination. See C. B. Translation.

* * In Russian.

[11] S. S. Salazkin, "Role of the liver in biosynthesis of urea in mammals" (Thesis, St. Petersburg, 1897). *

[12] M. V. Shepelev, "Collateral circulation in the portal vein system of the cat" (Thesis, Leningrad, 1952).*

[13] N. O. Yurinskii, "Pharmacology of ammonium chloride" (Thesis, St. Petersburg, 1894).*

[14] S. P. Bessman and A. N. Bessman, J. of Clin. Invest. v. 34, 622 (1955).

[15] S. P. Bessman, I. F. Fazekas and A. N. Bessman, Proc. Soc. Exper. Biol. a. Med., v. 85, 66 (1954).

[16] I. F. Fazekas, H. E. Ticktin and W. R. Ehrmantraut et al., Am. J. Med., v. 21, 843 (1956).

[17] E. V. Flock et al., J. Biol. Chem. v. 200, 529 (1953).

[18] R. D. Harkness, J. Physiol. v. 117, 287 (1952).

[19] W. V. McDermott, R. D. Adams and A. G. Riddel, Ann. Surg. v. 140, 539 (1954).

[20] H. L. Popper and N. C. Jefferson, Proc. Soc. Exper. Biol. a. Med., v. 85, 67 (1954).

* In Russian.