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CHANGES IN THE MACROSTRUCTURE OF THE BRAIN PROTEINS IN LIVING ORGANISMS IN CONNECTION WITH THE FUNCTIONAL STATE AND THE AMIDATION AND DEAMIDATION AS PROCESSES CAUSING THEM

Chair of Biochemistry
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The macrostructure of proteins is connected with the course of cell metabolism. The change in the spatial configuration of the macromolecules of proteins involves changes in their biochemical and biological activity including the catalytic activity of the ferments and their spatial distribution in the cells. This causes changes in metabolism and hence also in the corresponding physiological functions.

As it is generally known, one of the theories explaining the reaction of the cell to excitation is D.N.Nassonov's theory of denaturation. Starting on the one hand from the structurally immediate proximity of ammonia to protein, on the other hand from the facts on the connection of ammonia with the main physiological functions including the nervous processes (E.A.Vladimirova and others), we assumed by way of a working hypothesis (in 1957) that one of the biochemical factors of the changes in the protein macrostructure may be ammonia. The protein itself contains the simplest chemical mechanism for changing the macrostructure. It is the protein that is a carrier of ammonia in the form of reversibly splitting amide groups of dicarbonic acids, chiefly glutamic acid. In this case the relationship of ammonia to the main functional states of the brain-excitation and inhibition - is realized as a certain degree of the amidation of free carboxylic groups of proteins. This involves changes in the spatial configuration of the protein macrostructure on account of changes in the number and the position of free ionized carboxylic groups, i.e. in the number of free negative electric

charges of the macromolecule which may also influence the interaction between the carboxylic and other active protein groups (-SH). (Fig.1).

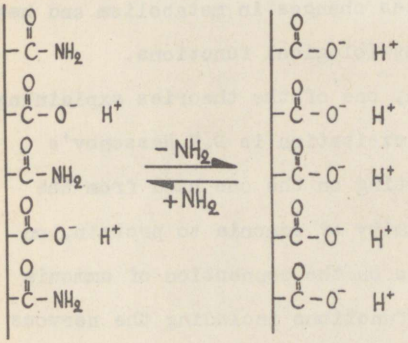

Fig.1. Scheme of amidation and deamidation of proteins.

Some other investigators (R.Vrba, Z.S.Gershenovitch) have also published data on the role of the brain proteins in the processes of ammonia formation and removal in the brain in connection with the functional state of the latter. However, to our knowledge, configurational changes in the brain have not been studied from this aspect.

We studied the following indices of the changes in the macrostructure of the proteins: electrophoretic mobility (established by means of paper electrophoresis), ultra-violet absorption spectra in the range of the wave-length 220-320 mμ and, in some cases, capacity for complex formation, i.e. the binding by proteins of S^{-35}-labelled methionine after A.G.Pasynyky.

We also determined the ammonia and glutamine content in the brain and the degree of the amidation of the proteins by means of protein-bound amide nitrogen.

Experiments were carried out on whole animals, chiefly on guinea-pigs and rabbits. In all the experiments the head was cut off at a definite moment and was immediately immersed in the liquid air for the freezing of the brain proteins for analysis.

With the aim of causing the amidation of the brain proteins, the accumulation of ammonia in the brain was carried out by a single or repeated parenteral administration of ammonia (NH_4Cl), by electric convulsions, by disturbance of urea synthesis by continuous drug-induced sleep or by means of experimental hepatitis or cirrhosis called forth by carbon tetrachloride.

In all these cases the level of ammonia (Fig.2), glutamine (Fig.3) and protein-bound amide nitrogen (Fig.4) rose in the brain, i.e. the degree of the amidation of the proteins increased.

In accordance with that in all these cases the rate of electrophoretic mobility of proteins was slowed down, caused by the binding of the free carboxylic groups by ammonia, which leads to a decrease in the total negative electric charge. And even the merging of some protein fractions was noticeable. (Fig.5). In the case of the single ammonia injection and electric convulsion, the total intensity of ultra-violet absorption spectra increased, which obviously represents the Rayleigh effect of dispersion reflecting the aggregation of the protein molecules as the subsequent stage of the primary changes in the structure of the protein macromolecules (Fig.6).

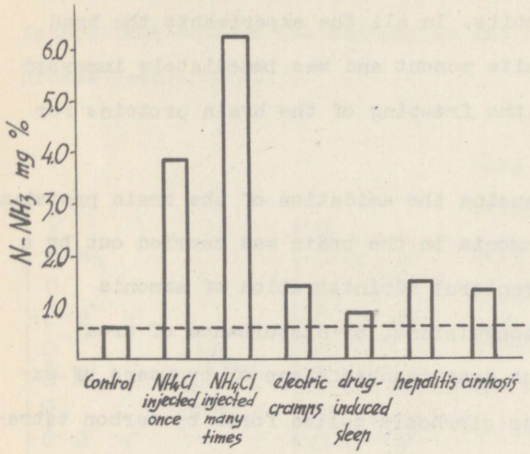

Fig.2. Ammonia content in brain under various conditions.

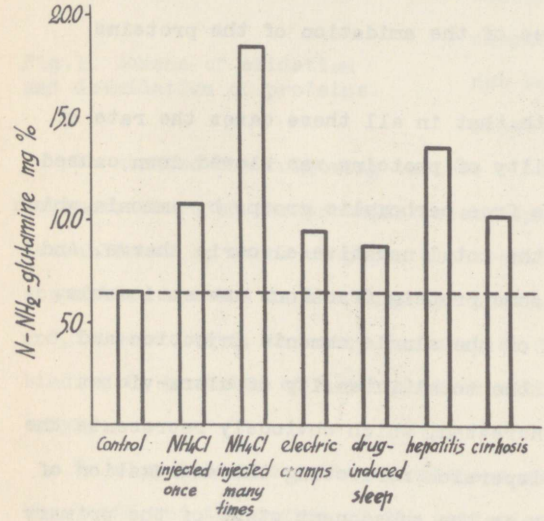

Fig.3.Glutamine content in brain under various conditions.

In continuous drug-induced sleep (of 2 weeks' duration)and the hepatitis and cirrhosis of the liver the intensity of the absorption spectra decreases (Fig.7), which gives evidence of a probably somewhat different character of changes in the macrostructure under the influence of the continuous action of ammonia on the brain proteins.

After the injection of ammonia (NH_4Cl) isolated pro-

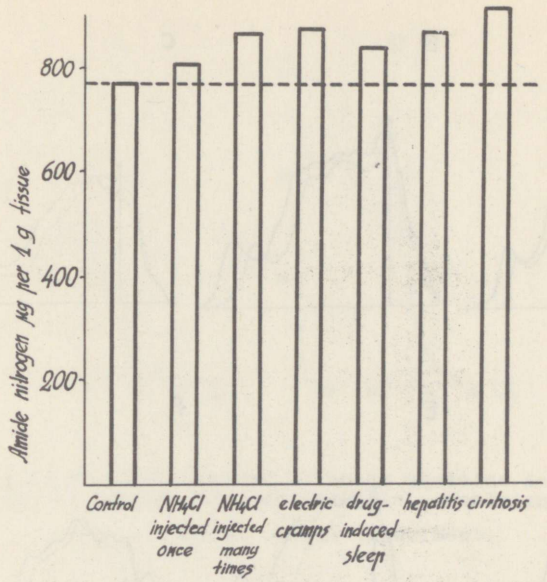

Fig.4. Increase in amidation of brain proteins under various conditions.

teins of the brain revealed a more intensive binding of S^{35}--methionine in vitro, which according to A.G.Pasynyky is a very sensitive test even of the initial stages of the denaturation changes in the proteins (Fig.8).

The reality of the changes discovered by us in the macrostructure of the brain proteins in the living organism was confirmed by similar changes in electrophoretic mobility (Fig.9) and in the absorption spectrum (Fig.10) after the parenteral administration of urea, a typical factor inducing the reversible denaturation of proteins in vitro. This is the more interesting that in their recent communication G.C.Stevenson et al.(1959), by means of electroencephalography, established

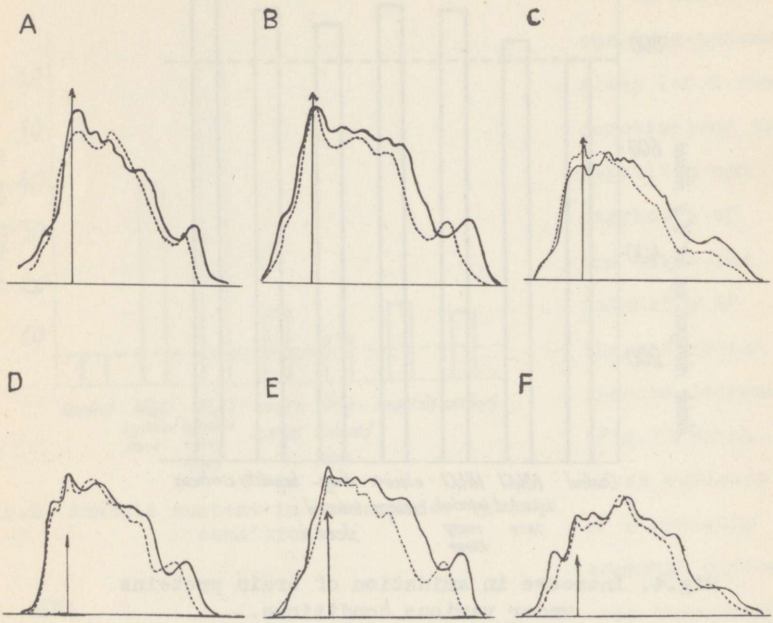

Fig.5. The rate of electrophoretic mobility of brain proteins under various conditions
A - single administration of NH_4Cl; B - repeated administration of NH_4Cl; C - electric convulsions; D - drug-induced sleep; E - hepatitis; F - cirrhosis; ——— control; ..... experiment

definite functional changes in the brain in urea injection as well as in uremia.

The reversibility of lifetime changes in the macrostructure of the brain proteins could be shown in experiments with

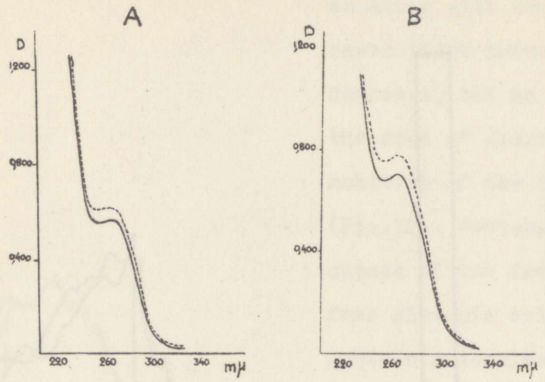

Fig.6. UV absorption spectra of brain proteins in the administration of ammonia (A) and electric convulsions (B)

——— control ..... experiment.

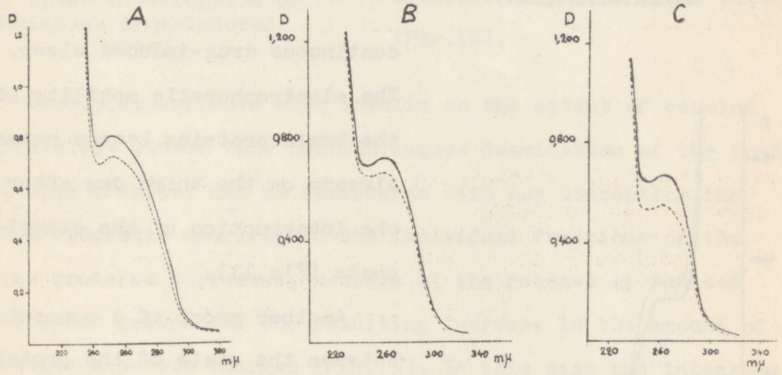

Fig.7. UV absorption spectra of brain proteins in drug-induced sleep (A), hepatitis (B) and cirrhosis (C)

——— control ..... experiment.

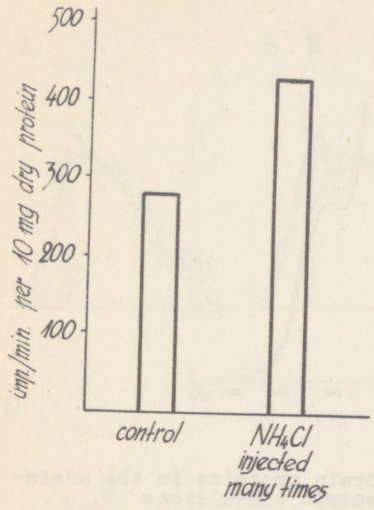

Fig.8.The binding of S^{35}-methionine by brain proteins after the repeated ammonia administration.

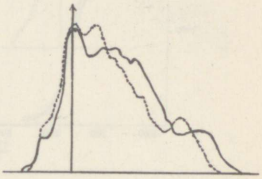

Fig.9.The rate of electrophoretic mobility of brain proteins after urea administration

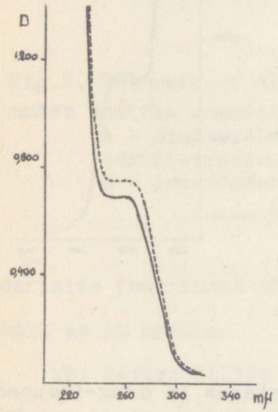

Fig.10. UV absorption spectrum of brain proteins after urea administration.

continuous drug-induced sleep. The electrophoretic mobility of the brain proteins became normal already on the third day after the interruption of the experiments (Fig.11).

Another proof of a connection between the state of the protein macromolecule and the amount of its free carboxylic groups are experiments with the injection of glutamic acid alone as well

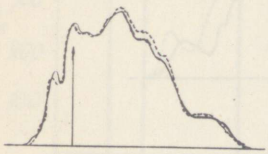

Fig.11. The rate of electrophoretic mobility of brain proteins on the 3rd day after interruption of continuous drug-induced sleep.

as along with ammonia. In both cases there proceeds not a decrease, but an increase in the rate of electrophoretic mobility of the brain proteins (Fig.12). Obviously in consequence of the inclusion of free glutamic acid in the protein molecules, increase in the amount of free carboxylic groups predominates over amidation. Here, too, a change in the intensity of ultra-violet absorption spectra takes place (Fig.13).

Lastly, experiments with insulin to the extent of causing convulsions showed that insulin causes deamidation of the brain proteins (Fig.14) and in accordance with our conception the electrophoretic mobility of the individual fractions of the brain proteins increases, because of the release of ionized carboxylic groups and the resulting increase in the amount of negative electric charges (Fig.15). In this case the intensity of the ultra-violet absorption spectra may increase as well as decrease (Fig.16).

Apparently the deamidation of the brain proteins provokes a change in the macrostructure of the brain proteins different from that of amidation. Inhibition of glutamine synthetase

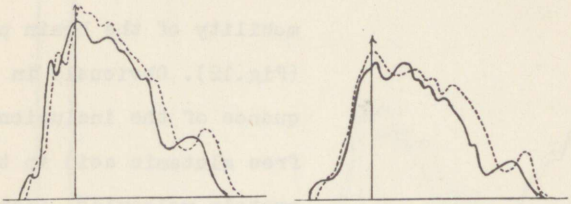

Fig.12. The rate of electrophoretic mobility of brain proteins after the administration of glutamic acid alone (A) and along with ammonia (B)
_____ control ..... experiment.

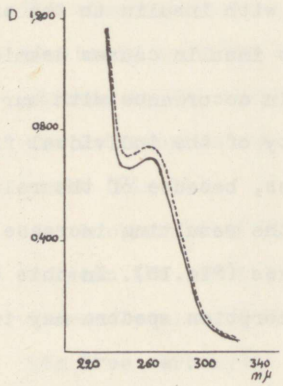

Fig.13. UV absorption spectrum of brain proteins after the administration of glutamic acid.
_____ control ..... experiment.

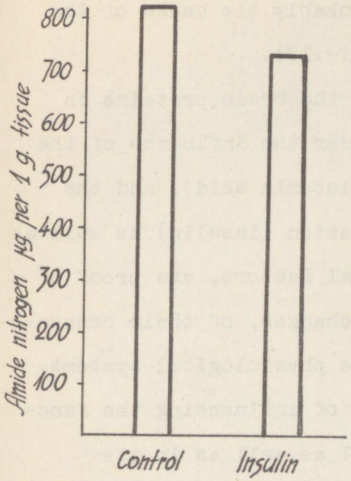

Fig.14.Decrease in amidation after administration of insulin.

Fig.15.The rate of electrophoretic mobility of brain proteins after the administration of insulin
_____ control ...experiment

Fig.16.UV absorption of brain proteins spectra after the administration of insulin
_____ control ..... insulin

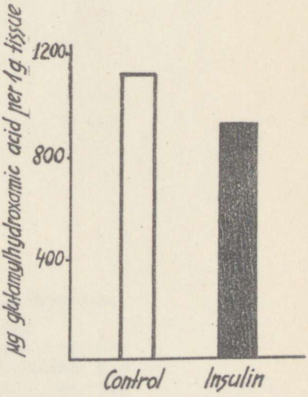

Fig.17.Activity of glutamine synthetase after the administration of insulin.

under the influence of insulin is probably the cause of the deamidation of the brain proteins (Fig.17).

Changes in the macrostructure of the brain proteins in living organisms which take place under the influence of the metabolic products (ammonia, urea, glutamic acid), and the hormonal factors of metabolism regulation (insulin) as well as under the action of drugs and physical factors, are proof of the "physiological" nature of these changes, of their connection with the functional state of the physiological systems. This opens up before the possibility of influencing the functioning of the organism in its normal as well as in its pathological state on the macromolecular level by changing the structure of the high polymers and the morphological cell structures formed by them by means of intermolecular bonds.

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ПРИЖИЗНЕННЫЕ ИЗМЕНЕНИЯ МАКРОСТРУКТУРЫ БЕЛКОВ МОЗГА В СВЯЗИ С ФУНКЦИОНАЛЬНЫМ СОСТОЯНИЕМ И АМИДИРОВАНИЕ И ДЕЗАМИДИРОВАНИЕ КАК ПРОЦЕССЫ, ИХ ВЫЗЫВАЮЩИЕ

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