THE ROLE OF THE PROTECTIVE AND TROPHIC SYSTEM OF ALVEOLI OF THE PIA MATER IN REMOVING AMINO ACIDS AND PROTEIN FROM THE CSF

N. A. Maiorova (Moscow)

A HISTOLOGICAL RADIOAUTOGRAPH STUDY

Laboratory of Experimental Neurohistology (Head – Corresponding Member AMN SSSR Prof. M. A. Baron), N. N. Burdenko Scientific Research Institute of Neurosurgery (Director – Active Member AMN SSSR B. G. Egorov), AMN SSSR Moscow
(Presented by Active Member AMN SSSR B. G. Egorov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 9, pp. 107-110, September, 1962
Original article submitted September 15, 1961

M. A. Baron [3] showed that the pia mater which lines the subarachnoid space is not uniform in structure. Two systems of canals and subarachnoid alveoli may be distinguished. The system of alveoli of the pia mater has a protective and trophic function, and maintains constant the composition of the CSF. Under pathological conditions this function may be considerably increased. In our previous investigation we studied the accumulation and the changes undergone in the endothelium of the alveoli of finely divided particles of brain substance introduced into the CSF of dogs [9]. In the endothelium of the alveoli we revealed histochemically the presence of neutral fats, fatty acids, phospholipids, and glycolipids.

The object of the present investigation has been to study the uptake and accumulation of amino acids and serum proteins into the alveoli. The following considerations have guided us:

1. Normally, the amount of amino acids and proteins in the CSF is very small, but they play a definite part in the metabolism of the endothelium of the pia mater, whose metabolism depends upon the CSF. 2. In pathological conditions, the amount of amino acids and proteins in the CSF increases considerably. They are to some extent eliminated by the flow of fluid out of the subarachnoid space [5, 13]. At the same time it must be admitted that the highly responsive system of alveoli is not indifferent to the enrichment of the CSF with amino acids and protein. It remains unexplained what part this system of alveoli plays in maintaining constant the composition of the CSF. 3. The protective role of the system of alveoli is brought about by the activity of its endothelium and by the formation by it of macrophages. A study of the part played by these and other cell elements in increasing the amounts of amino acids and protein in the CSF is required.

We have been able to find only a single work, that of Bowsher [11] dealing with this problem. Bowsher points out that protein is deposited in the pia mater from the CSF, but does not consider in what system of the membrane this process occurs, because he was not aware of the existence of these systems. Further, the method of histoautoradiography which he used was imperfect. He considered the artifact arising from the accumulation of grains of silver formed by interaction between the emulsion and the section as evidence of the presence of a radioactive substance. On this account his findings are unconvincing.

EXPERIMENTAL METHOD

We have introduced radioactive amino acids (methionine S^{35} and glycine C^{14}), and proteins (serum albumins and globulins) with subsequent histoautoradiography of the treated tissue. The serum proteins were labelled in a puppy in vivo by methionine S^{35}, and the albumin and globulin fractions were separated by electrophoresis of starch, and freeze-dried [5]. We carried out 18 experiments on dogs weighing 8-12 kg; we introduced methionine [8] and glycine [1] having an activity of 100 μCuries, albumins [3] and globulins [6] with an activity of from 3 to 10 microCuries, by the method of suboccipital puncture. The experiments lasted from 2 to $4\frac{1}{2}$ hours. After the dogs had been killed by the injection of 10% KCl into the heart, the meninges were removed and fixed in Carnoy's fluid. Paraffin sections 10 μ thick of the hemispheres, brain stem, cerebellum, and spinal cord with the membranes intact

were cut. Radioautographs of these sections were made by two methods on material obtained from the Scientific Research Motion Picture Institute; a type R emulsion was fixed on the sections, which were mounted on nuclear plates type MR [9]. The exposure lasted 3-7 days at 4° in the presence of anhydrous $CaCl_2$. An amidol developer was used, and fixation was in a 40% thiosulphate solution; the sections were stained in hemalum, and mounted in Canada balsam. As a control we used nonradioactive material treated in the same way. The radioautographs were studied microscopically, and attention was paid to the distribution and density of the β-tracks of S^{35} and C^{14} found above the different structures of the meninges.

At the end of each experiment we carried out a paper electrophoresis of the CSF, and made radioautographs of the electrophoregrams; we also precipitated the protein of the fluid with a 10% solution of trichloracetic acid, and then counted the activity of the protein precipitate and of the supernatant fluid. We found that the labelled proteins were still labelled in the fluid. We therefore attributed the radioactivity of the structures in the radioautographs entirely to their uptake of the injected proteins.

EXPERIMENTAL RESULTS

On the whole there was agreement between the results obtained by microscopic examination of the radioautographs after the injection of either amino acids or proteins. Most of the tracks were found above the endothelium of the walls of the subarachnoid alveoli. In places where the alveoli were denser, for example in the sulci of the cerebral hemispheres, the density of the β-tracks was greater. With an immersion objective, it could clearly be seen that β-tracks lay over the cytoplasm of the endothelium of the alveoli, and radiated out chiefly from the endoplasmatic zone. Each territory of cytoplasm surrounding isolated nuclei or groups of nuclei of the endothelium formed on the average, 2-4 tracks in the emulsion (Fig. 1).

The results obtained show that the labelled amino acids and proteins are eliminated from the CSF rapidly, in 30 min after the injection, and are taken up by the endothelium of the protective and trophic system of the subarachnoid alveoli. There is no doubt that the process is responsible for the uptake by the endothelium of these two different kinds of proteins are not the same. We may suppose that the amino acids are involved in the cytoplasmic metabolism of the endothelium, and become incorporated into the proteins of the cytoplasm during the renewal process [12]. The protein fractions, like other colloids injected into the CSF, accumulate in the "vacuum" in the cytoplasm. The protein then becomes concentrated in the cytoplasm of the endothelium from the surrounding fluid. This strongly-shown property of the endothelium to take up substances in this way also underlies the protective and trophic function of the subarachnoid alveoli, whose activity maintains constant the composition of the CSF.

The power of the endothelium of the subarachnoid alveoli to take up methionine and protein (especially the latter) shows a considerable resemblance of this tissue to the reticular tissue of the lymphatic nodes. The latter also take up methionine and labelled proteins intensively. Labelled proteins retained in reticular tissue of the lymphatic nodes are known to be antigens stimulating the formation of antibodies [11, 14, 15].

An important part in the protective and trophic function of the system of subarachnoid alveoli is played by the macrophages. This function usually arises and develops later than the reaction of the endothelium. In our short-term experiments we could observe only the initial phase of the reaction. In smears of CSF removed from dogs at the end of the experiment we found a small number of macrophages and large mononuclear cells which had become separated from the endothelium of the walls of the alveoli. Historadioautographs showed that these cells and others left β-tracks on the photographic emulsion, indicating the presence in these cell forms of labelled proteins. Judging by the large number of β-tracks arising from each macrophage, it must be inferred that the free macrophages take up proteins more vigorously than does the alveolar endothelium. These facts agree well with the findings of B. V. Kedrovskii [6, 7], who pointed out the role of the tissue macrophages in the transformation of protein.

It is important to note that no radioactive protein was ever found in the neutrophils which entered the subarachnoid space from the blood after the injection of protein into the CSF.

The radioautographs showed that the endothelial lining of the blood vessels of the pia mater, which resemble the endothelial wall of the alveoli, also take part in the uptake of amino acids and proteins from the CSF. In this connection, it is interesting that methionine was taken up not only by the endothelial lining of the vessel, but also more strongly by the muscular tunic of the arteries. Protein, on the other hand, accumulated more densely in the endothelial linings, and only a small amount penetrated into the thickness of the vascular walls (Fig. 2).

A study of the arachnoid membrane, either on film preparations or on sections showed that the β-tracks leaving it are more or less evenly distributed over the surface. They can be related neither to any particular structural ele-

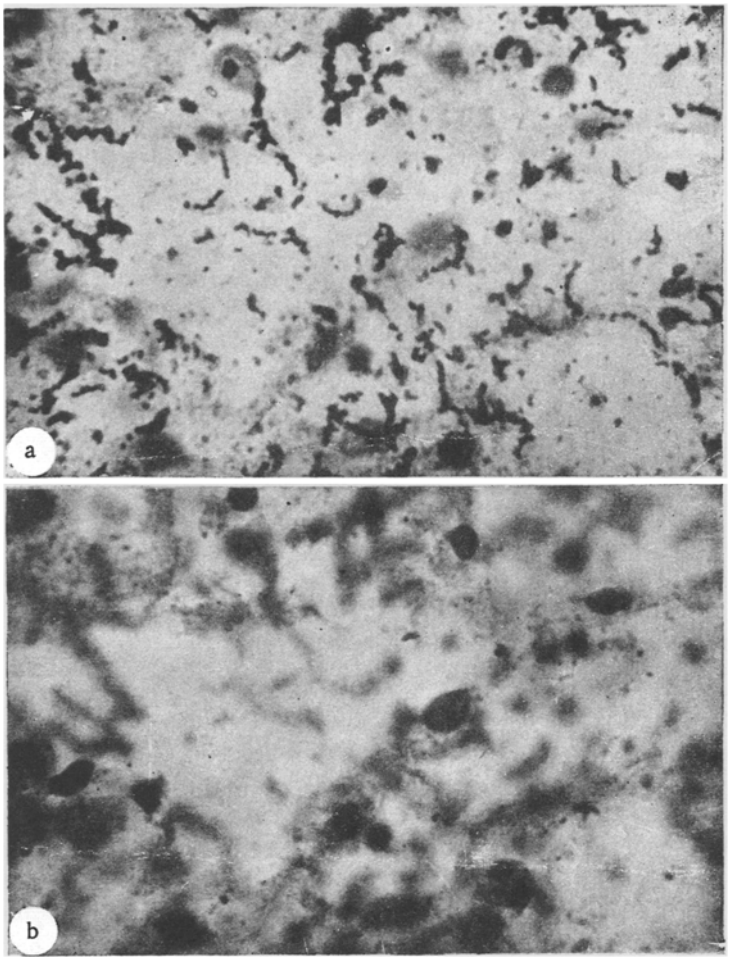

Fig. 1. Changes in the tissues 30 days after the introduction of India ink; no phytoncides used. Early stages of formation of a fistula along the thread; necrosis and infiltration by polymorphs. (Ocular 7 ×, objective 10 ×; stain hematoxylin-eosin).

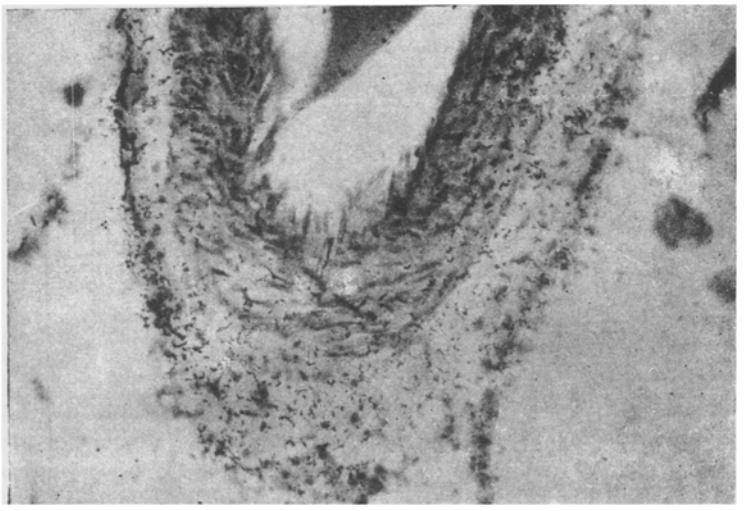

Fig. 2. Changes in the tissues 30 days after the introduction of India ink; phytoncides used. A fistula has been found, lined with stratified squamous epithelium (ocular 7 ×, objective 10 ×; stain hematoxylin-eosin).

ments of the membrane, nor, in particular, to cells of the external covering layer. The tracks may be due either to the uptake of amino acids and protein fractions into the cells of the arachnoid, or to movement of the fluid containing these substances through it, because it is known that the passage of the CSF through the arachnoid mater is the principal means whereby it is removed from the subarachnoid space [1, 4]. It is however possible that both actions occur. There is also an undoubted uptake of amino acids and proteins into the "cell spots" formed by the outer covering layer of the arachnoid membrane. Within these "cell spots" a clearly-marked accumulation of β-tracks can be seen in the emulsion. Apparently the vigorous uptake of amino acids and protein into the "cell spots" is associated with the fact that these structures are proliferating regions with a metabolic rate, and represent the source from which the villi and arachnoid granulations develop [2].

We found no differences between the uptake of albumins and globulins. It took place at the same rate at all times during the experiments.

SUMMARY

Labeled amino acids (methionine S^{35} and glycine C^{14}) and labeled blood serum protein fractions (albumins, globulins, methionine-S^{35} labeled) were administered into the subarachnoidal space in 18 experiments on dogs. Distribution of amino acids and proteins in various structures of the pia mater was ascertained by histoautoradiography. Only a few hours after their introduction, the amino acids and proteins were found in the cytoplasm of the endothelium of the subarachnoid cells, which constitute a protective and trophic system of the pia mater. Amino acids and proteins were also taken up by the endothelial lining of the blood vessels in the subarachnoid space, which were related to the endothelium of the subarachnoid cells. The uptake of amino acids and proteins into the endothelium represents one of the principal factors for maintaining constant the composition of the cerebrospinal fluid in pathological conditions.

LITERATURE CITED

1. I. A. Alov, Byull. éksper. biol., Vol. 25, No. 3 (1948), p. 227.
2. M. A. Baron, Reactive Structures of the Inner Meninges [in Russian], Leningrad (1949).
3. M. A. Baron, Transactions of the 1st All-Union Conference of Neurosurgeons, Leningrad (1958), p. 24.
4. M. A. Baron, F. M. Lyass, and N. A. Maiorova, Vopr. neirokhir., No. 1, Leningrad (1959), p. 3.
5. M. A. Baron, Blood-brain Barriers [in Russian], Moscow (1961), p. 322.
6. B. V. Kedrovskii, Uspekhi sovr. biol., 20, 1, 41 (1945).
7. B. V. Kedrovskii, The Cytology of Protein Syntheses in the Animal Cell [in Russian], Moscow (1959).
8. G. S. Lykova, É. I. Shcherban', and L. V. Funshtein, A Textbook of Historadioautography [in Russian], Leningrad (1957).
9. N. A. Maiorova, Vopr. neirokhir., No. 6 (1961), p. 8.
10. F. Haurowitz, W. Friedberg, H. Walter et al. In book: Contributions to the International Conference on the Peaceful Uses of Atomic Energy, [Russian translation], Moscow (1958), vol. 12, p. 639.
11. D. Bowsher, Anat. Rec., Vol. 128 (1957), p. 23.
12. J. Brachet, Biochemical Cytology [Russian translation], Moscow (1960).
13. F. C. Courtice and W. J. Simmonds, Aust. J. exp. Biol. med. Sci., Vol. 29 (1951), p. 255.
14. A. H. Coons, In book: Structural Aspects of Cell Physiology. Symposia of the Society for Experimental Biology No. 6, Cambridge (1952), p. 166.
15. J. H. Humphrey, Proc. roy. Soc. B., Vol. 146 (1956), p. 34.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
