# Chronobiological studies on the blood-brain barrier[1,2]

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*Summary.* Horseradish peroxidase (HRP) is a useful tracer for a study of the transportation of exogenous materials across vascular walls. After i.v. administration of HRP, reaction products of HRP can be recognized in the granular perithelial cells of small cerebral blood vessels at particular times of day. It suggests that there is a time-dependent fluctuation in the permeability of small cerebral blood vessels.

During an investigation of cerebral small vessels, the authors noticed specific perithelial cells which were provided with large, round fluorescent inclusion bodies; some of these findings are reported in previous papers[3-5]. Recently, it was also demonstrated that the granular perithelial cells (FGP) were able to incorporate exogenous proteins administered intraventricularly[3].

It has been known for a long time that a rigid barrier exists between blood vessels and cerebral parenchyma, and that macromolecules such as ferritin and horseradish peroxidase (HRP) do not normally penetrate across the barrier, and moreover, that the impediment is based on the occurrence of tight junctions developed between 2 successive endothelial cells[6]. However, in 1973, Westargaard and Brightman[7] presented evidence that the blood-brain barrier is not absolutely established and in some segments of cerebral arterioles, exogenous protein (HRP) is transferable to extravascular spaces. In 1980, Petito and Levy[8] pointed out the significance of arterioles in alterations of the blood-brain barrier under some experimental conditions. In this paper, the permeability of cerebral small vessels was examined with an estimation of the reaction product of HRP incorporated in FGP at different times of day.

*Material and methods.* The animals employed here were 24 Wistar male rats weighing 270-300 g. Before use, they were kept under standardized environmental conditions including temperature (24 ± 1 °C), food and water ad libitum, and an artificial light and dark cycle (light period 08.00-20.00 h, dark period 20.00-08.00 h) for 3 months. The rats were anesthetized slightly with ether and injected with 0.5 ml of physiological saline containing 15 mg of horseradish peroxidase (HRP, Sigma Chemical, Type II) in a great saphenous vein every 4 h (01.00, 05.00, 09.00, 13.00, 17.00 and 21.00 h). 30 min after injection, they were killed by decapitation and the parietal region of the cerebral cortex was sliced with a blade in cold physiological saline under a binocular microscope. Half of the sliced specimens were stretched[9] and fixed with paraformaldehyde gas at 40-60 °C for 3 min. Those specimens were treated with diaminobenzidine[10] for visualizing the reaction product of HRP. The other half of the specimens were fixed with 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.4) and cut with a Vibratom (Oxford) at a thickness of 50 µm, and then they were treated with the diaminobenzidine method[10]. The specimens were fixed again in Millonig solution[11] and embedded in epon 812. After cutting the specimens with a Porter-Blum MT-2B ultramicrotome, thin sections were stained with uranyl acetate and lead hydroxide and used for electron microscopical observations. Thick sections and stretched specimens were employed for counting labeled granules (reaction product of HRP) in FGP. This experiment was performed in the spring and in the summer of 1980.

Number and intensity of labeled granules in FGP at different times of the day

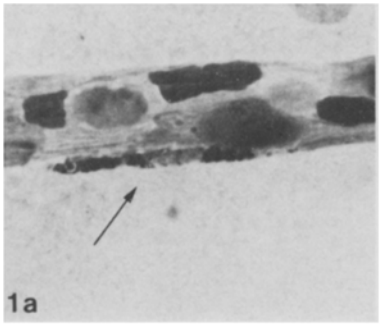
| Clock time (h) | Number of FGP | Number of labeled granule in FGP | | | Granule per FGP |
|---|---|---|---|---|---|
| | | High density | Low density | Total | |
| 01.00 | 55 | 99.0 | 89.5 | 188.5 | 3.43 |
| 05.00 | 55 | 237.5 | 50.5 | 288.0 | 5.24 |
| 09.00 | 55 | 8.0 | 71.5 | 79.5 | 1.45 |
| 13.00 | 55 | 1.5 | 14.0 | 15.5 | 0.28 |
| 17.00 | 55 | 7.5 | 60.5 | 68.0 | 1.24 |
| 21.00 | 55 | 59.5 | 64.0 | 123.5 | 2.25 |

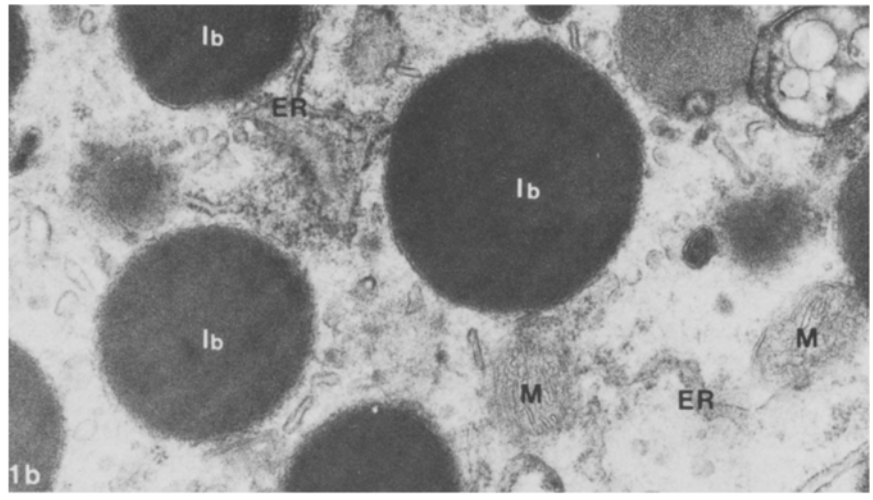
The value in each item is the average of measurements on 4 rats.

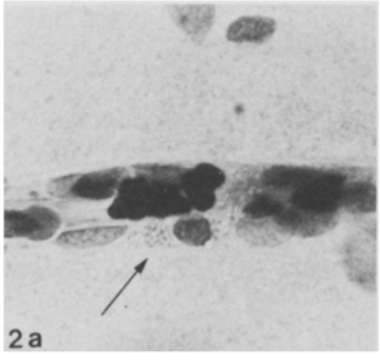
*Results.* Figures 1a, 2a and 3a were light micrographs showing FGP and intracellular labeled granules at 05.00, 13.00 and 21.00 h. The reaction product of HRP appeared as brownish granules at a light microscopic level. The quantitative change of labeled granules in FGP was as follows; at 01.00 h, a fairly large amount of deposit was observed in FGP; about half of the granules were intensely labeled, and the others less so. At 05.00 h, labeled granules increased and most of them were markedly intense. The number and intensity of deposits reached a peak at this time. But at 09.00 h the intensity of labeled granules reduced and the proportion of slightly stained granules increased. At 13.00 h, reaction products diminished significantly and could hardly be seen in FGP with a light microscope (fig. 2a). Then, at 17.00 h, some reaction positive granules came out again and at 21.00 h, a certain amount of labeled granules appeared (fig. 3a). That is, in the daytime the deposits were less, while at night they grew in number and intensity. The fluctuation in intensity and number of labeled granules is summarized in the table. In order to visualize smaller reaction products in FGP, the electron microscope was employed. Figures 1b, 2b and 3b are electron micrographs of FGP and inclusion bodies at 05.00 h (fig. 1b), 13.00 h (fig. 2b) and 21.00 h (fig. 3b). Comparing the specimens, it was ascertained that the number and intensity of reaction positive deposits varied with clock time at the electron microscopical level too. Figure 1b showed intensely dark inclusion bodies and vesicles, while in figure 2b, the electron opacity of inclusion bodies was reduced and they looked pale. Only some vesicles were labeled deeply. In figure 3b, inclusion bodies were moderately intense and labeled vesicles were recognized around them.

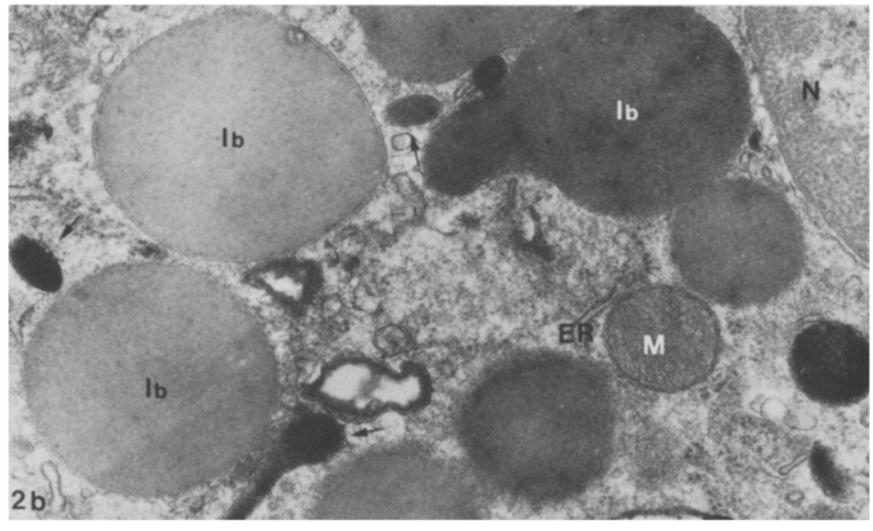
Summarizing the findings mentioned above, there appears to be maximal permeability in the vascular wall at 05.00 h (which is towards the end of the dark / active phase in the rats), and a minimum at 13.00 h (which is the midnight / sleep phase) in spring and summer.

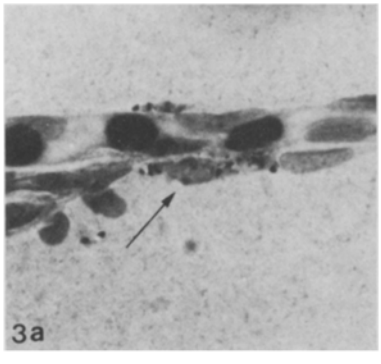
There are 2 possible ways of explaining these alterations of the deposit in FGP. The one is concerned with the daily fluctuation in the uptake capacity of FGP, and the other is associated with that of the permeability of vascular walls. The permeability may include vesicular transport in arterioles as Petito and Levy[8] suggested. However, according to this authors' experience, the substance administered intraventricularly is transferable into FGP at any time, and the uptake capacity of FGP appears to keep a constant level through a day. The changes are therefore due to changes in the permeability of the vascular walls of cerebral small vessels at different times of day.



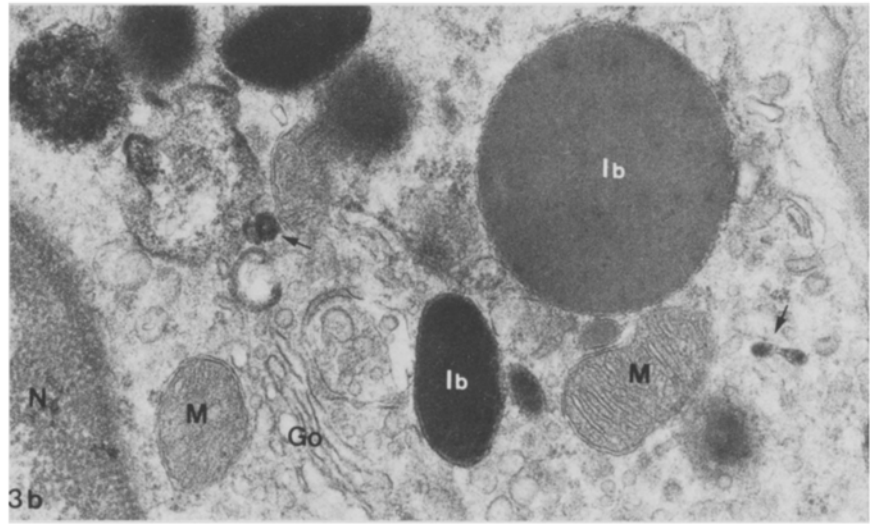




Figure 1. *a* 05.00 h, light micrograph. The slender FGP is filled with reaction products of HRP (arrow). Red cells are also labeled intensely owing to the presence of hemoglobin. ×680. *b* Electron micrograph. Inclusion bodies (Ib) and vesicles are markedly intense. ER; endoplasmic reticulum, M; mitochondrion. ×26,000.

Figure 2. *a* 13.00 h, light micrograph. No labeled granules can be seen in FGP (arrow). ×680. *b* Electron micrograph. Pale and less intense inclusion bodies (Ib) are seen. The electron opacity of small ones is rather intense (arrows). ER; endoplasmic reticulum, M; mitochondrion, N; nucleus. ×26,000.

Figure 3. *a* 21.00 h, light micrograph. Several labeled granules are scattered in FGP (arrow). ×680. *b* Electron micrograph. Large inclusion body with a moderate electron opacity and intense small granules (arrows) are depicted. Go; Golgi apparatus, Ib; inclusion body, M; mitochondrion, N; nucleus. ×26,000.

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## Failure of somatostatin to influence experimental tumor cell growth in vivo and in vitro

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*Summary.* The influence of somatostatin on tumor cell growth was studied in vivo in mice (sarcoma 180 ascites tumor and Lewis lung tumor) and in vitro on nontransformed and polyoma-transformed cell lines. 4 or 20 µg/100 g of cyclic somatostatin and 4 µg/100 g of linear protamin Zn-bound somatostatin were injected s.c. twice daily in the in vivo study. Cyclic somatostatin (1, 4 or 10 µg/ml) was added twice daily to the cell cultures. Somatostatin administration influenced neither the survival of animals nor the growth rate of cultured cell lines.

It has been established by many workers that somatostatin exerts an inhibitory action on various endocrine and exocrine functions[1]. In addition, it has been demonstrated that this tetradecapeptide inhibits endotoxin-induced leukocytosis and growth of granulation tissue in man and animals[2], gastrointestinal cell proliferation[3], and cell-free ribosomal protein synthesis[4,5] in rats. In a previous study we observed an inhibitory effect of somatostatin on the release of colony stimulating activity from mouse spleen lymphocytes in vitro[6]. From these results we considered the possibility that somatostatin could act as a direct inhibitor of cell proliferation. In this study we investigated the antitrophic action of somatostatin on experimental tumor cell growth in vivo and in vitro.

*Material and methods.* 60 male C57B1 mice, 6-8 weeks old, were injected i.p. with sarcoma 180 ascites tumor cells. 60 male $DFB_1$ (DBAxC57B1) mice received i.m. Lewis lung tumor cells. The animals were divided into groups of 15 (4

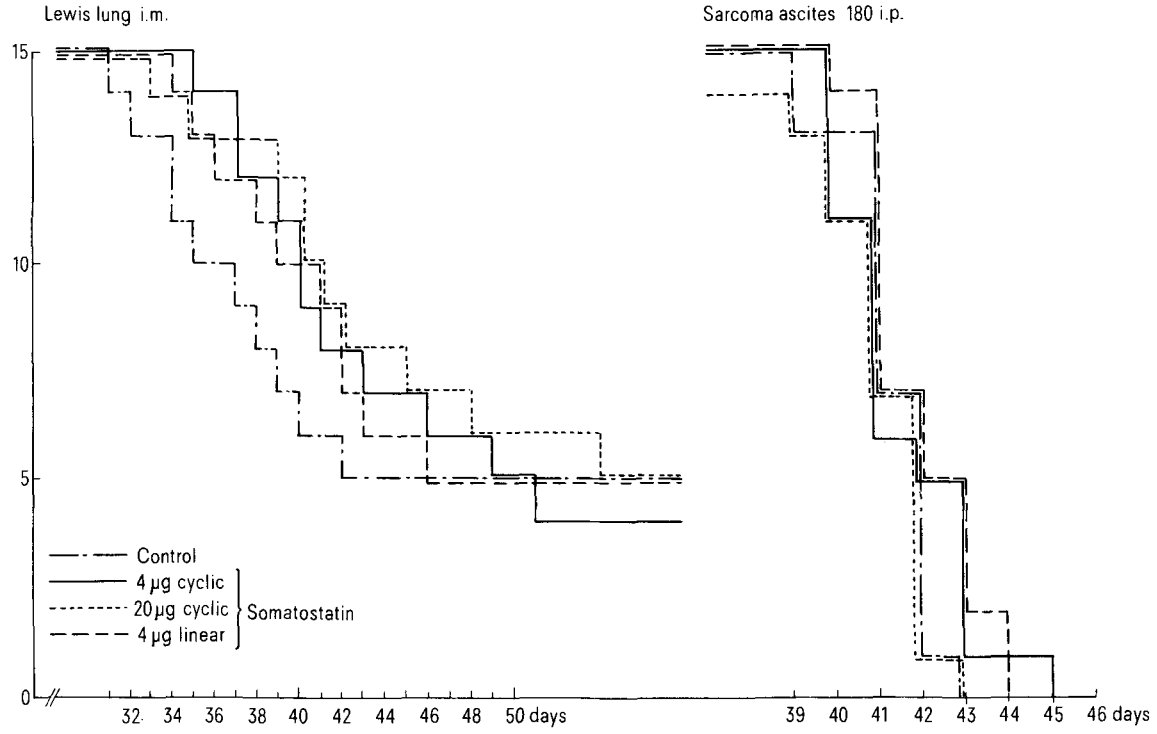


Figure 1. Effect of different doses of somatostatin on the survival of mice injected with either sarcoma 180 ascites tumor (right) or Lewis lung tumor (left). Ordinate: Number of animals alive. Abscissa: Days from tumor injection.