Reticulocytes continued to incorporate radioiron during the entire incubation period. Addition of exogenous rat erythropoietin to the incubation media did not produce any alteration in reticulocyte iron incorporation. However, the addition of LT 3 or DT 3 in concentrations of 0.1 μg/ml of incubation media produced an increase in the iron incorporation of the reticulocyte. Both LT 3 and DT 3 produced similar increases in reticulocyte iron incorporation. These results indicate that erythropoietin and triiodothyronine have different mechanisms by which erythropoiesis is stimulated. In addition, the erythropoietic stimulation produced by LT 3 and its calorigenically inactive dextroisomere was similar.

Zusammenfassung. Verabreichung von D-, wie auch von L-Triiodothyrosin erzeugt eine ähnliche Vermehrung der Erythropoiesis in nephroektomierten Ratten, wie bei der Messung der Radioeiseninkorporation durch Erythrozyten (18 h nach Injektion von radioaktivem Eisencitrat). Es ergab sich weiter, dass D- und L-Thyrosin, im Unterschied zu Erythropoieten, die reticulozytose Eisenassimilation in vitro vermehrte. Danach scheinen verschiedene Mechanismen bei der Stimulation der Erythropoiesis durch Erythropoieten und Triiodothyronin im Spiele zu sein.

R. M. DONATI, M. E. JOHNSON, C. A. STANCER and LW. R. STROMBERG

Department of Radiation Biology, Division of Nuclear Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington (D.C. 20012, USA), 8 December 1967.

The Effect of Nickel Chloride on the Permeability of the Blood-Brain Barrier

According to the observation of EHRLICH in 1885[1] certain aniline dyes do not penetrate into the nervous system, although they stain other organs of the organism. This observation was the basis of the blood-brain barrier theory. At present, the blood-brain barrier is considered to be a multiplex mechanism regulating the exchange of certain metabolites by decreasing or enhancing transport between blood vessels and brain tissue[2-4]. Different experimental or pathological effects may cause a selective damage in some functions of the barrier[5].

Electron microscopic investigations have shown[6,7] that the cytoplasm of the endothelial cells of the capillaries in the central nervous system is continuous (not fenestrated) and surrounded by a well developed basement membrane. Between the layers of the basement membrane, pericytes and their processes are localized. Externally, glial end feet adhere to the capillaries. The electron histochemical investigations of TORACK and BARRNETT[8] indicate that there is a 5-nucleoside phosphatase activity in the glial elements and the basement membrane. In other organs and in capillaries of brain areas not protected by the barrier (choroid plexus, area postrema), nucleosid phosphatase activity can be found in the pinocytotic vesicles of the endothelial cytoplasm[9].

In this paper the question will be dealt with whether there is a correlation between the ultrastructural localizations of 5-nucleoside phosphatase in brain capillaries and the permeability of the haematoencephalic barrier. Alterations in the permeability of the blood-brain barrier were studied after specific inhibition of nucleosid phosphatase.

Our studies were performed on albino rats weighing 150–250 g. For the specific inhibition of the 5-nucleoside phosphatase, the nickel chloride inhibition technique described by KAYE[10] was used. 0.025 g/kg and 0.5 g/kg nickel chloride was administered i.p. to 25 rats. Subsequently, 5–20–60 min or 2–5–10 h, respectively, 2–5 ml of 1% trypane blue (in physiological saline) as barrier test material was injected into the tail vein of the animals. The animals were decapitated after 5–10–20 min, and 20 μ thick parasagittal sections were obtained from the vermis of the cerebellum by means of a kryostate (type Mirköz). The distribution of the dye was examined by the fluorescent method of HAMBERGER and HAMBERGER[11]. The sections were embedded in Entellan and examined with a Zeiss fluorescence microscope, using a high pressure mercury vapor lamp and BCr-12 and OG1 filters.

Following an i.v. injection of trypane blue, a wide range of capillaries can be seen under the fluorescence microscope (Figure 1). In the course of fluorescent examinations performed 30 min after the administration of 0.15 g/kg nickel chloride, it was striking that the trypane blue showing a characteristic red fluorescence leaked out of the capillaries, resulting in an intensive red perivascular fluorescence. Later the leakage of the dye increased considerabely, thus 20, 120 min after the administration, the fluorescent dye exuded from most of the capillaries and diffused profoundly into the cerebral tissue (Figures 2 and 3). However, inspite of the enzyme inhibition, the dye did not leak out of a few capillaries even at this stage. Trypane blue injected 6–10 h after administration of nickel chloride could always be detected within the vascular bed.

Our results confirm the investigations of TORACK and BARRNETT[8] according to which 5-nucleoside phosphatase plays and important role in the regulation of the permeability conditions in the capillaries of the central nervous system. According to the above authors, in the capillaries of the brain areas protected by the hematoencephalic barrier, 5-nucleoside phosphatase is not localized in the cytoplasm of the endothelial cells, but in the basement membrane and in glial elements. On the basis of the localization of the 5-nucleoside phosphatase in the basement membrane and in glial end feet, BARRNETT[12] assumes that

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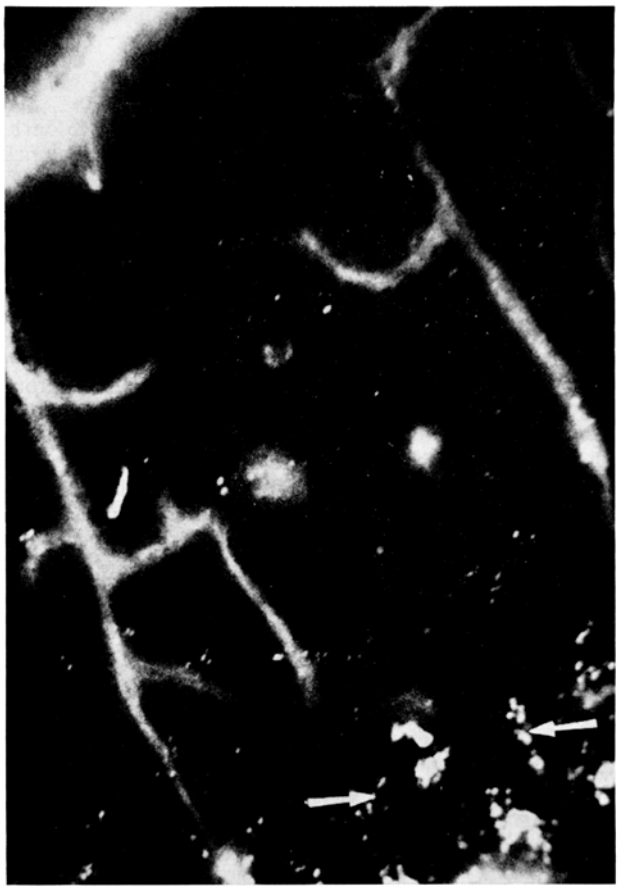

Fig. 1. Fluorescence in the molecular layer of the rat cerebellum after i.v. injection of 1% trypane blue. The wall of the capillaries shows sharp contours, dye leakage cannot be detected. Autofluorescent lipofuchsin pigment granules are marked by arrows. × 700.

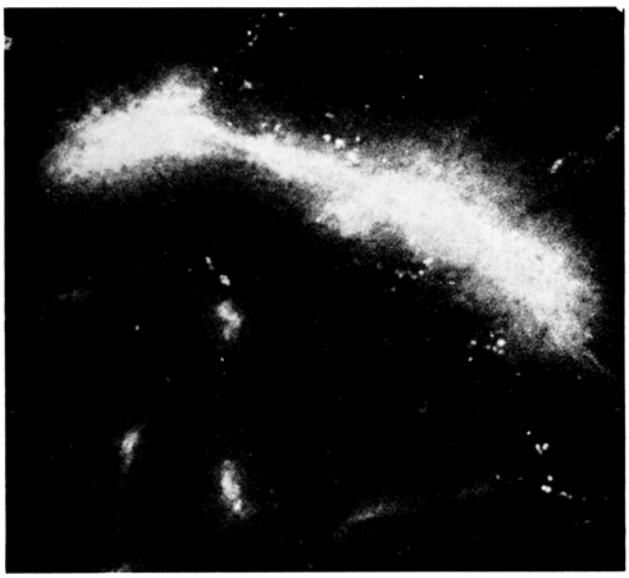

Fig. 2. Fluorescence due to 1% trypane blue injected i.v. 60 min after i.p. administration of 0.15 g/kg nickel chloride. Molecular layer of rat cerebellum. Perivascular fluorescence points to the enhancement of capillary permeability. In the lower part of the picture, capillaries showing intact contours are visible. × 700.

Fig. 3. Trypane blue injected 120 min after the inhibition of 5-nucleoside phosphatase leaked out of the capillaries and diffused profoundly into the cortex of the cerebellum. In the lower right hand corner of the picture, the autofluorescent lipofuscin granules of Purkinje cells can be seen. × 700.

these structures exert no barrier function, but promote an active transport. Our investigations, however, suggest that the basement membrane and the glial end feet form a barrier for the penetration.

GRIEG and HOLLAND[13] observed an increased permeability of the hematoencephalic barrier after injection of acethylcholine. In a previous paper[14] we reported a close topographic histochemical correlation between butyryl cholinesterase activity of capillaries and the function of the hematoencephalic barrier. Ontogenetical studies on the butyryl cholinesterase activity of brain capillaries showed[15] that the enzyme activity of the capillary wall becomes complete in the same period when the hematoencephalic barrier develops, i.e. during the third week of postnatal life. The present enzyme histochemical and dye penetration studies show that the enzymes demonstrated in the cerebral capillaries play a part in the regulation of the permeability of the hematoencephalic barrier.

Further morphological investigations concerning the significance of the different enzymes participating in the function of the blood-brain barrier are in progress.

Zusammenfassung. Die Permeabilitätsverhältnisse der Blut-Hirn-Schranke nach spezifischer Hemmung der 5-Nucleosid-Phosphatase wurden mittels Fluoreszenzmikroskopie studiert und eine Permeabilitätserhöhung nach Injektion von Nickelchlorid gefunden: Trypanblau kann dabei ins Gehirn penetrieren, was dafür spricht, dass die 5-Nucleosid-Phosphatase bei der Regulation der Kapillarpermeabilität eine bedeutende Rolle spielt.

T. VÁRKONYI and F. JOÓ

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