from those in the normal adult. The lymphoid aggregates contained numerous reactive cells, but insufficient examples were included in cryostat sections to be certain that the staining was always identical to that in adult Peyer's patches.

Mesenteric and peripheral lymph nodes were hypoplastic in the germ-free rats and similar to the rudimentary development in new-born and young animals. Comparative histochemical data are incomplete, however, because positive specimens for frozen sections were not obtained from all germ-free animals. Only trace reactions for GAL and GLCR were present in the specimens available; this degree of staining was identical to the reactions in lymph nodes from young animals. In conventionalized animals, mesenteric nodes were well developed and the follicles contained germinal centers, but there was great variation in the maturation of peripheral lymph nodes. In contrast to the marked reactivity in normal adult lymph nodes, those from conventionalized adults showed only moderate enzyme staining. Reactive mononuclear cells were seen in medullary cords, peripheral sinusoids, and less commonly, around follicles.

There were mononuclear cells in the lamina propria of bronchial mucosa in germ-free animals. Peribronchial lymph nodes were hypoplastic or absent, and no lymphoid aggregates were found adjacent to peripheral bronchioles. Changes in each were readily apparent in the conventionalized animal. The histochemical reactions reflected the differences. As in neonatal specimens, only trace or absent staining was found in the lungs of germ-free animals, but moderate numbers of reactive cells were present in the bronchial lamina propria and lymphoid nodules of conventionalized and normal adult specimens.

The above findings confirm the correlation between morphologic maturation and histochemical reactivity for GAL and GLCR in lymphoid tissues. They also show that the relatively hypoplastic organs and reduced levels of activity for those enzymes in germ-free animals are similar to the morphology and staining of neonatal tissues. Likewise, the appearance and reactions in specimens from conventionalized and normal adults is similar, although the changes in the spleen and lymph nodes appear to be retarded. It is hazardous to interpret histochemical data too closely, but the observed differences were usually marked and consistent. Furthermore, indolyl reactions have been shown to be highly specific and relatively semiquantitative[5].

Numerous investigators have used gnotobiotic animals for experimental studies, especially with respect to the structure and function of the reticuloendothelial system. Although phagocytic and opsonic activities in germ-free and control rats are comparable[6]; reduced digestive capacity of macrophages has been demonstrated in germ-free animals[7] and is of particular relevance to the present observations because GAL and GLCR are representative acid hydrolases.

It is significant that the most marked differences were found in the thymus and lung, since the new-born thymus is functionally isolated from antigen and the lung of germ-free animals is exposed only to well-filtered air. In contrast, the less marked difference in intestinal mononuclear cells may reflect the diet of germ-free animals, which is free of bacteria, but not devoid of foreign material[8].

For these reasons, we interpret the similarity between germ-free and neonatal lymphoid tissues in reactivity for galactosidase and glucuronidase as additional evidence for the role of antigen in the normal neonatal development of lysosomal enzyme activity.

*Zusammenfassung.* Die Ergebnisse histochemischer Färbeverfahren für β-Galaktosidase und β-Glukuronidase in lymphoiden Geweben von keimfreien Ratten sind ähnlich denjenigen von neugeborenen Tieren. Diese Daten stellen einen zusätzlichen Beweis für die wichtige Rolle der Antigene bei der normalen neonatalen Entwicklung der lysosomalen Enzymaktivität dar.

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## Investigation of the Blood Brain Barrier Permeability in Experimental P Avitaminosis

The basis of the blood brain barrier theory was Ehrlich's observation made in 1885 that certain anilin dyes do not pass from the blood stream into the central nervous system whereas they stain other organs. In modern times the blood brain barrier may be regarded as a complex mechanism regulating the transport of certain metabolites between the blood vessels and the nervous tissue. Various pathological and experimental effects can cause selective damage in the function of the barrier.

In an earlier paper[1] we reported that in experimental P avitaminosis (in rats kept on SHERMAN-LAMER-CAMPBELL's[2] diet) we found significant and pronounced pathological anatomical changes, a significant increase in cerebral edema and subpleural hemorrhages with simultaneous decrease of capillary resistance. Treatment with bioflavonoids obviated these changes in a statistically significant way.

The aim of our present work was to study the behavior of the blood brain barrier in rats kept on SHERMAN-LAMER-CAMPBELL's diet containing no bioflavonoids. Male rats of the R-Amsterdam strain weighing 180 ± 20 g were used for our investigations. The animals were given standard compressed food for 2 weeks, then for 2 months they were kept on SHERMAN-LAMER-CAMPBELL's deficiency diet[2]. In the 8th week blood brain barrier test material, 1% Evans blue solution (in physiological saline) 15 ml per kg of body weight was injected into the tail vein of the animals according to the method of HAMBERGER and HAMBERGER[3]. 15 min after administration of the dye the animals were decapitated and 20 μm thick

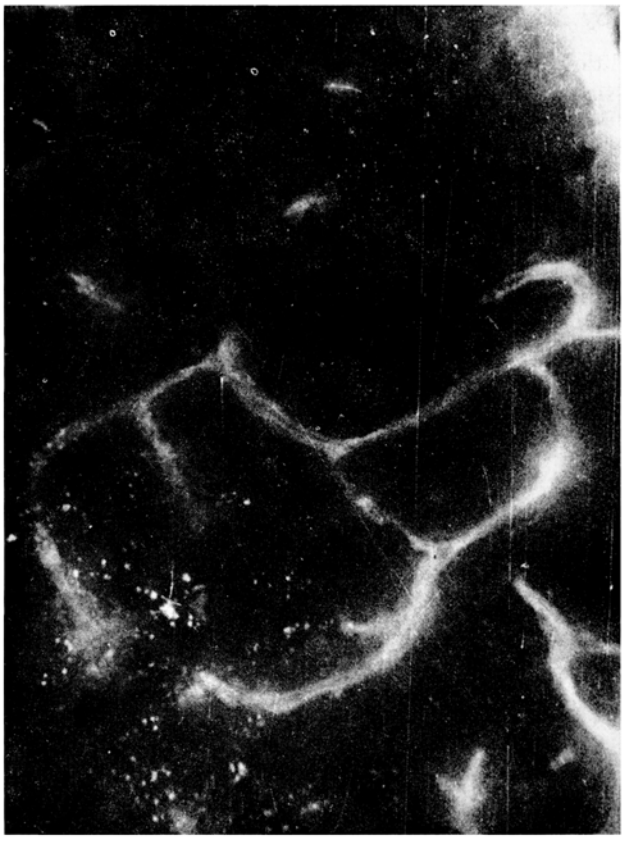

Fig. 1. Fluorescence in the molecular layer of the rat cerebellum after i.v. injection of 1% Evans blue. The wall of the capillaries shows sharp contours, dye leakage cannot be detected. ×622.

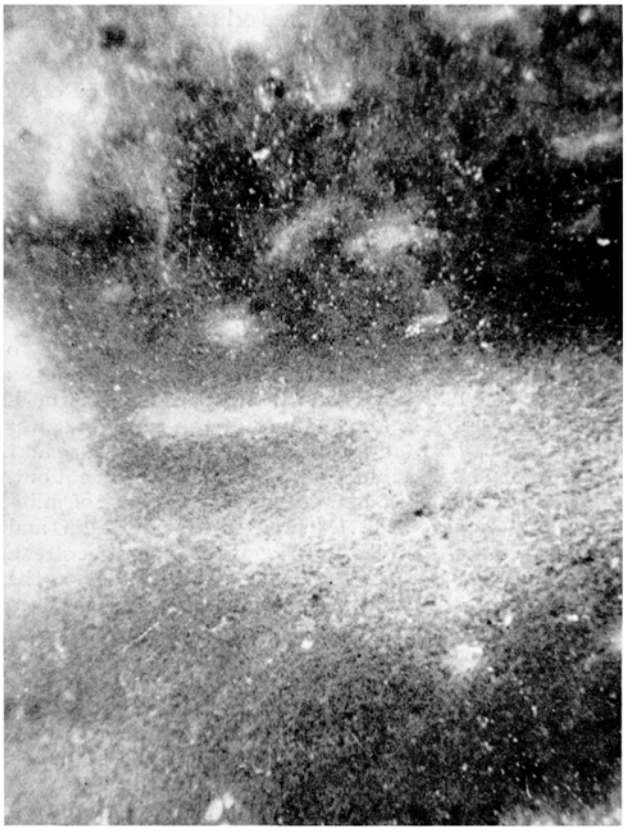

Fig. 2. Fluorescence due to 1% Evans blue in the molecular layer of rat cerebellum. Perivascular fluorescence points to the enhancement of capillary permeability. ×444.

parasagittal sections were made from the vermis of the cerebellum with the aid of Kryostat. The sections were embedded in Entellan and examined under a Zeiss fluorescent microscope with the help of a mercury vapor Lamp (HBO-50) and an OGI filter. Rats kept on normal diet provided controls. In the control animals many capillaries fluorescing in characteristic red light could be seen following the injection of Evans blue (Figure 1). Red fluorescence was a sign that the dye did not leave the blood vessels owing to the function of the blood brain barrier. In certain areas in the so-called circumventricular organs, an example of which is the plexus chorioideus, where the blood brain barrier does not act against the dye, very intense diffuse red fluorescence was seen in the parenchyma. In rats kept on SHERMAN-LAMER-CAMPBELL's diet, 15 min after administration of the dye, the characteristic fluorescence of Evans blue could be seen even perivascularly in a very pronounced form staining the parenchyma surrounding the blood vessels (Figure 2). As the dye does not leave the blood vessels in the cerebral areas protected by the hematoencephalic barrier, the diffusion of the dye and its degree indicate damage of the barrier. Thus, on the basis of our investigations we may say that in the central nervous system of the animals kept on SHERMAN-LAMER-CAMPBELL's deficiency diet capillary permeability is abnormally increased.

Since RUSZNYÁK and SZENT-GYÖRGYI[4] considered the vitamin nature of citrin as proven, they named it vitamin P on account of its effect enhancing capillary permeability. There are still many who dispute even now the vitamin nature of the flavonoids[5,6] and it seems that we still know too little about the biological effects of the flavons. Comparing the literary data with our fluorescence examinations we see that SHERMAN-LAMER-CAMPBELL's flavone-deficient diet influences the central nervous system. This influence manifests itself in the abnormally increased permeability of the capillaries functioning here.

*Zusammenfassung.* Eine erhöhte Permeabilität der Blut-Hirn-Schranke für Evans-Blau wurde bei Ratten nach einer Flavon-Mangeldiät festgestellt.

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