about 1 month after the appearance of the tumor. All the others were killed at the end of 9 months after the first pellet implantation. At autopsy, the body and organ weights were measured and the right thoracic mammary gland was used for a whole mount preparation.

Results and discussion. The results are illustrated in the Table. There was no difference among groups in the body weight change. The anterior pituitary and ovarian weights had a trend to decrease in groups I and II as compared with group III, although the difference was not statistically significant except the difference between groups II and III in the ovarian weight ($P < 0.01$). These results agreed with the authors' previous results[3]. The mammary tumor appearance was significantly inhibited by ergocornine or CB-154: Only 10 and 20% in groups I and II of mice respectively had a tumor at the end of the experiment, whereas mammary tumors appeared in 74% of mice in group III. At autopsy, no pellets could be found in the mice with tumors as well as in a few others of groups I and II. These pellets may have been lost on account of different causes. The whole mount preparations of the mammary glands in these mice showed rather marked lobuloalveolar development similarly to that in group III, while in this respect the glands of the other mice of groups I and II were in an almost complete rudimentary state. In good agreement with the authors previous results[3], the mammary glands in groups I and II had scarce HN, while there existed numerous huge HN in the gland of group III. All the results indicate that these ergot alkaloids inhibit the spontaneous mammary tumor appearance in mice and that this inhibitory effect is based on their suppression of the development and growth of mammary HN.

Zusammenfassung. Die subkutane Impantation von Ergocornin oder 2-Br-α-Ergokryptin (CB-154) hemmt die Bildung von spontanen Tumoren der Milchdrüse bei der Maus.

R. YANAI and H. NAGASAWA[5]

Pharmacology Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo (Japan),
18 February 1971.

[5] The authors are indebted to Drs. M. TAESCHLER and E. FLÜCKIGER, Sandoz Ltd., Basel, for their kind donation of ergocornine methanesulfonate and CB-154.

Lysosomal Hydrolases in Lymphoid Tissue from Germ-free and Neonatal Rats

The development of indolyl methods for the histochemical demonstration of enzyme activity by HOLT[1] and PEARSON[2] has provided an improved technique for the study of lymphoid tissue[3]. In a previous report, we have applied these methods to the neonatal reticuloendothelial system and found that, in general, the intensity and pattern of staining was correlated with morphologic maturation[4]. There were two exceptions: reactivity in the thymus was delayed, but in contrast, staining of mononuclear cells in the intestinal lamina propria preceded the normal neonatal proliferation and differentiation of these cells. The antigenetic isolation of the thymus and the heavy exposure of the intestine to foreign material suggested that antigen 'proximity' might be important to the maturation of degradative enzymes in lymphoid tissues. The following experiment was designed to test the hypothesis since the exposure of germ-free animals to foreign material is greatly diminished.

The thymus, spleen, proximal jejuneum, distal ileum, lung, and when identifiable, mesenteric, axillary, and popliteal lymph nodes were removed from a group of 6 sacrificed adult germ-free Sprague-Dawley rats and from normal new-born, 7-day- and 14-day-old animals. Tissues from normal adult and germ-free animals which had been conventionalized for 60 days were used for comparison. All specimens were quickly frozen in a dry ice-acetone bath and stored at −70 °C. Frozen sections were subsequently prepared and incubated with the indolyl galactoside and glucuronide, cleared through xylol, and mounted according to the methods described previously[2,5]. Portions of each tissue, or in the younger animals, from litter mates, were used for paraffin sections.

The histologic appearance of the thymus from the germ-free rat was similar to that from the new-born animal in that the cortex was imcompletely developed. Sections incubated for galactosidase (GAL) and glucuronidase (GLCR) showed occasional, faintly reactive cells in the medulla and cortex. The level of staining was identical in neonatal specimens, but markedly reduced in comparison to the normal adult. In the thymus from conventionalized rats, the cortex was somewhat thicker and numerous mitoses were present. The histochemical reactions were similar to those in normal adult rat specimens. Reactive cells appeared to be medium and large lymphocytes which were otherwise indistinguishable from surrounding cells.

The spleen from germ-free animals had an unusual appearance. The follicles were small and contained no germinal centers, and the marginal zone was broad, but poorly demarcated. As in the neonatal specimens, trace or absent staining was formed with both substrates. The marginal zone was still indistinct in conventionalized animals, but there were numerous germinal centers. Large mononuclear cells reactive for GAL and GLCR were found adjacent to the follicles and scattered in the red polp. The staining intensity was somewhat less than in the normal adult, and most similar to 14 day specimens.

The small intestine in germ-free animals was dilated, and the normal villi were blunted. There was a paucity of reticular cells in the lamina propria and small, infrequent lymphoid aggregates. Although reduced in total number, a large proportion of the interstitial cells stained intensely for GAL and GLCR; the reactivity was similar to week-old rather than a new-born specimen. In conventionalized animals, interstitial cells were more numerous; Peyer's patches were smaller than in the normal adult, but there were frequent follicles with germinal centers. The histochemical reactions for both enzymes were indistinguishable

[1] S. J. HOLT and J. F. DANIELLI, *General Cytochemical Methods* (Academic Press, New York 1958), p. 375.
[2] B. PEARSON, P. L. WOLF and J. VAZQUEZ, Lab. Invest. *12*, 124 (1963).
[3] B. PEARSON, A. C. STANDEN and J. R. ESTERLY, Experientia *23*, 954 (1967).
[4] J. R. ESTERLY, A. C. STANDEN and B. PEARSON, Lab. Invest. *21*, 497 (1969).
[5] J. R. ESTERLY, Nature, Lond. *216*, 821 (1967).

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.

J. R. ESTERLY and A. C. STANDEN

University of Chicago, Pathology Department, 950 East 59th Street, Chicago (Illinois 60637, USA) and Bjarne Pearson Medical Sciences Laboratory, Fort Detrick, Frederick (Maryland, USA),
13 November 1970.

[6] T. M. SABA, J. P. FILKINS and N. R. DILUZIO, Proc. Soc. exp. Biol. Med. *125*, 634 (1967).
[7] H. BAUER, F. PARONETTO, W. A. BURNS and A. EINHEBER, J. exp. Med. *123*, 1013 (1966).
[8] Y. B. KIM, S. G. BRADLEY and S. W. WATSON, J. Immun. *97*, 52 (1966).

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