from the *Kalanchoe* tumors, although capable of rapid growth in the presence of the inciting bacteria, have little endoplasmic reticulum (Figure 4), and closely resemble *Kalanchoe* cells stimulated to rapid growth by exogenously applied auxin[5].

Other workers have demonstrated that meristematic cells, which are believed to actively secrete auxin, are similary rich in endoplasmic reticulum[9,10]. These observations support the hypothesis that extensive endoplasmic reticulum is characteristic of auxin-producing cells and is not a cellular response to auxin.

The important role of rough endoplasmic reticulum in protein synthesis in plant and animal cells has been extensively documented. An abundance of endoplasmic reticulum in protein-secreting plant cells has also been reported[11]. This suggests that protein synthesis and auxin synthesis may have a common subcellular site[12].

Résumé. Le cytoplasme des cellules végétales capables de synthétiser l'auxine est caractérisé par l'abondance du réticulum endoplasmique. Les cellules dont la croissance est stimulée par de hautes concentrations de cette hormone contiennent beaucoup moins de réticulum endoplasmique. D'où l'hypothèse que le lieu de synthèse de l'auxine (dans les cellules capables de cette synthèse) est peut-être le réticulum endoplasmique.

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Inhibition by Ergocornine and 2-Br-α-Ergocryptin of Spontaneous Mammary Tumor Appearance in Mice

Hyperplastic nodules (HN) are represented as the preneoplastic state of spontaneous mammary tumor development in mice[1]. The authors previously reported that ergocornine and 2-Br-α-ergokryptin (CB-154) suppress the pituitary prolactin secretion and inhibit the development and growth of mammary HN in mice[2]. Furthermore, they have demonstrated that these ergot alkaloids inhibit the prolactin secretion not only from the in situ but also the grafted pituitaries[3]. The present experiment was carried out in order to investigate whether or not ergocornine and CB-154 suppress the appearance of spontaneous mammary tumors in mice when administered continuously for a long period as pellet.

Materials and methods. Animals used were 3- to 5-month-old virgin mice of C3H/He strain bred in the authors' laboratory. They were divided into 3 groups. Groups I and II were implanted subcutaneously with pellets of ergocornine methanesulfonate and CB-154, respectively. Each ergot alkaloid was thoroughly mixed with cholesterol in the ratio of 1:4, and 50 mg of the mixture was pelleted in the size of 5 mm in diameter and 2 mm in thickness. Group III as the control received the same size of pellet of cholesterol only.

Approximately 4 weeks after the pellet implantation, all mice were given 1 pituitary isograft each under the right kidney capsule expecting the enhancement of mammary tumor development[4]. Four months after the first pellet implantation, each group received one additional pellet of the same drug. All mice were examined for palpable mammary tumors once a week throughout the experiment. The mice with palpable tumors were killed

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Body and organ weights and mammary tumor incidence in each group

Group	Treatment	No. of mice	Body weight (g)		Change (%) (%)	Anterior pituitary weight (mg)	Ovarian weight (mg)	Mammary tumor incidence (%)
			Initial	Final				
I	Ergocornine	10	23.0 ± 0.7[a]	32.4 ± 0.9	43 ± 6	1.98 ± 0.10	11.1 ± 1.9	20.0[b] (2/10)[d]
II	CB-154	10	22.1 ± 0.5	33.1 ± 1.5	50 ± 8	2.11 ± 0.12	8.7 ± 0.7[c]	10.0[c] (1/10)
III	Control	19	22.3 ± 0.6	32.1 ± 0.6	43 ± 5	2.30 ± 0.09	14.8 ± 1.2	73.7 (14/19)

[a] Mean ± standard error of the mean. [b] Significant against control $P < 0.02$. [c] Significant against control $P < 0.01$. [d] No. of mice with tumors/total No. of mice examined.

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.

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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

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