were removed and their wet weight determined. The adrenal glands were fixed in Bouin's solution for 24 h. Subsequently they were embedded in methacrylate. The histological sections which were then prepared were 4 µm thick and were stripped with AR 10 Kodak film. The exposure lasted 30 days. For the analysis of the histoautoradiograms 3000 cells were randomly selected from each of the 3 cortical zones (z. glomerulosa, z. fasciculata, z. reticularis) from both adrenal glands. The ^{3}H-thymidine labelled cell nuclei and mitoses were identified. The results obtained formed the basis for the calculation of the ^{3}H-index (number of labelled nuclei per 1000 nuclei) and the mitotic rate (number of mitoses per 1000 nuclei).

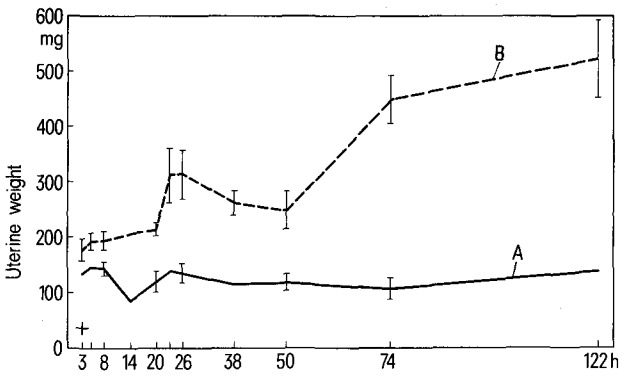

Figure 1. Time dependent increase in uterine weight; A, control group; B, oestradiol group.

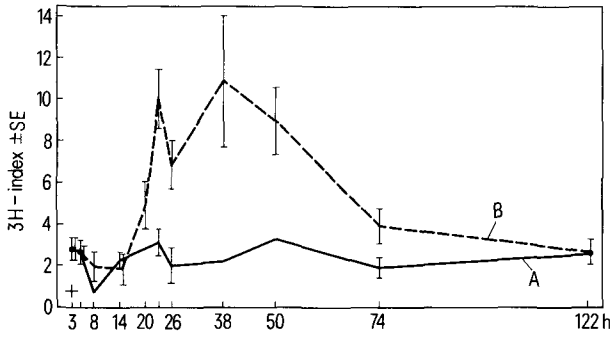

Figure 2. DNA-synthesis in the cells of the zona glomerulosa of the adrenal gland of female rats after ovariectomy and application of oestradiol; A, control group; B, oestradiol group

Results. The effect of oestradiol administered to a castrated female rat can be seen from the state of the uterus[8–10]. Like these authors we observed a continuous increase in the weight of the uterus (fig. 1). After 14 h the weight of the uteri in the treated group was significantly greater than that of the control group. This increase had not yet reached a maximum by the time scheduled for ending the investigation, namely 122 h. In contrast, the ovarian hormone had no influence whatsoever on the weight of the adrenal glands.

In this study the action of oestradiol lasted between 3 and 122 h. Particular attention was given to the investigation of the kinetics of proliferation in the zones of the adrenal cortex following ovariectomy and a single administration of ovarian hormone in relation to time. An unequivocal result was observed only in the zona glomerulosa. About 14 h after the administration of oestradiol an increase in the DNA synthesizing cells began to take place in this zone and this reached a maximum at 38 h. Thereafter a decrease in the DNA synthesis occurred. At the end of the investigation the values in the treated animals were the same as those obtained in the controls (fig. 2). An almost comparable influence was observed on the rate of mitosis in the cells of the zona glomerulosa. No significant changes in the ^{3}H-Index or in the Mitotic Rate were found in the zonae fasciculata and reticularis.

In order to clarify these results, further studies are in progress to determine whether the changes observed are the result of a direct effect following the administration of ovarian hormone or whether they are exerted via the anterior lobe of the pituitary gland.

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Ultrastructural changes in uterine myometrium of mice with experimentally-induced adenomyosis[1]

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Summary. Ectopic pituitary transplantation induced a high incidence of adenomyosis in SHN mice. Early signs of the development of adenomyosis were the penetration of stromal connective tissue into myometrium followed by uterine gland invasion. Associated with these changes, the inner layer of myometrium showed the involution of smooth muscle cells and distended intercellular spaces.

Key words. Mouse, uterine myometrium; uterine myometrium, mouse; adenomyosis; ectopic pituitary transplantation; prolactin.

We have recently found that prolactin plays an important role in the development of adenomyosis, a pathological state of the endometrial tissues, in mice[2,3]. As observed under the light microscope, the myometrium became loose by experimentally induced hyperprolactinemia, which causes the development of adenomyosis. After the disturbance of arrangement of the muscular layer, endometrial tissues of both glandular and stromal components penetrated through the myometrium, culminating in adenomyosis[4]. Huseby and Thurlow[5] also indicated that in mice chronic hyperprolactinemia is a major endocrine factor in the genesis of adenomyosis. In this study, the ultrastructural changes of the myometrium after pituitary grafting into the uterus, which induces rapid and high incidence of adenomyosis[2], was examined.

Fifty-day-old female mice of the SHN strain received a single isologous anterior pituitary each in the lumen of the right uterine horn and were killed 14 weeks after the grafting. Control mice were given a piece of submaxillary gland each at the same sites and killed at the corresponding ages. Number of mice examined was 5 in each group. At autopsy, the middle portion of the right uterine horn was fixed in Bouin's fluid. Paraffin sections of the tissue were stained with hematoxylin and eosin for light microscopy. For electron microscopy, thin sections of the uterine tissue, fixed in glutaraldehyde-paraformaldehyde, post-fixed in osmium tetroxide, and embedded in epoxy resin, were stained with uranyl acetate and lead citrate.

In light-microscope observations, adenomyotic changes were found in all mice given the pituitary grafts. Early morphological disorder in the development of adenomyosis was a penetration of the stromal connective tissue into the myometrium followed by uterine gland invasion throughout the stromal penetration. Associated with these changes, the myometrium, especially the inner layer of the smooth muscle bundles arranged concentrically around the long axis of the uterine horn, became loose as a whole, and contained the cells with scanty cytoplasm (fig. 1), as has been described in a previous paper[4]. In contrast, the uterus of control mice with grafts of submaxillary glands did not show any adenomyotic changes. The myometrium was composed of tightly arranged muscle cells. These observations suggest again that changes in the myometrium may be responsible for the occurrence of adenomyosis. In electron-microscope observations, the most striking changes of myometrium in mice showing adenomyosis was the involution of the muscle cells of the inner layer bundles. The cells reduced their size to about one-third and the intercellular spaces were distended as compared to those of control mice showing no adenomyotic uterine changes (fig. 2). In control mice, the cells of the inner layer of the smooth muscle bundles were apposed with narrow intercellular spaces and, in some part, showed much interdigitation of the cell membranes (fig. 3). The myometrium of mice with adenomyosis frequently showed penetration of the stromal connective tissue in the distended intercellular spaces and disintegration of muscle cells with lysosomes in their scanty cytoplasm. Disintegration of muscle cells was hardly observed in the myometrium of the control mice. In addition, the number of pinocytotic vesicles (pits), which are characteristic structures of the plasma membrane of smooth muscle cells, was smaller in the muscle cells of mice with adenomyosis than in the controls. However, in the former, a number of vacuoles larger than pits was observed in the peripheral cytoplasm and their nuclei frequently contained strongly condensed heterochromatin. Other cytoplasmic organelles such as mitochondria with a dense matrix, short cisternae of granular endoplasmic reticulum, Golgi bodies, 2 types of myofilaments, dense bodies and free ribosomes in the perinuclear region, were not noticeably different between the muscle cells of experimental and control mice. Marked changes such as those observed in the inner muscle layer were not evident in the outer layer of muscle cells arranged parallel to the long axis of the uterine horn.

Thus it is evident that these changes such as involution and disintegration of the muscle cells and wider intercellular spaces in the inner layer of the myometrium are responsible for easy

Figure 2. The inner layer of uterine myometrium of a SHN mouse which received a pituitary isograft and was killed 14 weeks after the grafting. Note involution of muscle cells, distended intercellular spaces and invasion of stromal connective tissue (SC). × 5046.

Figure 1. The uterus of a SHN mouse which received a pituitary isograft and was killed 14 weeks after the grafting. Note invasion of endometrial tissues into connective tissue space (SP) between inner and outer layers of uterine myometrium. The inner layer of myometrium (M) contains the smooth muscle cells with scanty cytoplasm. × 120.

Figure 3. The inner layer of uterine myometrium of a SHN mouse which received a piece of submaxillary gland and was killed 14 weeks after the grafting. The muscle cells are apposed with narrow intercellular spaces and contain a number of pinocytotic vesicles on the plasma membrane. × 5046.

invasion of endometrial tissues into the musculature. In rats, prolactin enhances the response of uterus to trauma or decidual formation[6,7], causes the acceleration of estrogen binding to uterine explants[8] and exerts a marked inhibitory effect on oxytocin-induced contraction of uterine myometrium primed with estrogen and progesterone[9]. Furthermore, prolactin binding is found in rat uterus[10]. Although a target tissue of prolactin in the uterus has not yet been determined, the present findings may involve a possible effect of chronic stimulation of prolactin on the smooth muscle cells.

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Comparison of wing margin structures of *vestigial* and wild-type *Drosophila melanogaster* grown at 31 °C

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Summary. The development of the middle row of triple row wing bristles was examined in flies homozygous for the *vestigial* mutant, grown at 31 °C. While bristle number, length and spacing all improved toward the wild-type condition, the mean values for both sexes were only about 10% of those of wild-type, although development in males was significantly more complete than in females. The results suggest an explanation for the apparent lack of regenerative ability in this tissue.
Key words. Vestigial; imaginal disc; regeneration.

Many studies[1-4] have shown that the length, structure and cell death rate in the development of the wings of *Drosophila melanogaster* vary significantly with developmental temperature in animals homozygous for the mutant *vestigial* (2-67.0). At restrictive temperatures, the mutant gene causes the failure of the development of the distal ¾ of the wing, including all parts of the wing margin. Restrictive temperatures are considered to be 25 °C and below, while permissive temperatures were established by Stanley[1] at 30 °C, and more recently by Bownes and Roberts[2] at 29 °C. While studies of other aspects of wing disc development have shown patterns more nearly approaching those of wild-type at 31 °C[5], the extent of development of structures in the adult wing of *vestigial* flies raised at 31 °C has not been reported. The capacity of the wing disc to regenerate the distal part of the wing in *vestigial* flies cannot be determined until the development of distal wing structures at 31 °C has been analyzed. All flies used in this study were raised at 31 °C. Survival at this temperature is reduced by approximately 20% from normal levels, but no distinction was seen in survival rates for the two sexes. Two coisogenic stocks were used, derived from Oregon R wild-type and *vg* in an Oregon R background. We selected one distal wing structure for examination; the central row of bristles in the triple row (TR), characteristing the leading edge of the wing. This row of bristles is ideal for examination in both normally developed wings and in those developing from transplanted discs. In addition, it lies in a part of the wing particularly sensitive to the effects of the vestigial mutation[6]. Both wings from each of a minimum of 30 flies in each experimental group were removed and mounted in Euparal for examination. The number of bristles was determined, and the length of the middle row of the TR was measured through a 25 × ocular with a micrometer. Statistical comparisons were made by standard methods.

Results. We examined wild-type and *vestigial* flies of both sexes, and compared the two wings on each fly. No significant differences were found between the means of right and left wing measurements, and variation between sides in wild-type flies was extremely small. Greater differences were seen in *vestigial* flies of both sexes, although both sides were affected equally often.

Vestigial animals grown at 31 °C in our cultures produced three distinct categories of flies: those with at least part of the TR formed on each wing, those with TR on one wing only, and those with no TR on either wing. The results are shown in the table.

Vestigial males produced TR structures on one side only in 9 out of 74 cases (12.2%). Females developed unilateral TR structures in 8 out of 52 cases (15.4%). When the data for paired (bilateral) and unpaired (unilateral) TR structures were compared in females, no significant differences were found in the three parameters measured. In the case of males however,

Condition of middle row bristles of the wing margin's triple row (TR) at 31 °C

		Length of bristle row (μm)	Number of bristles	Interbristle distance (μm)
Wild-type	Females	1175 ± 47.8	71.2 ± 3.6	16.5 ± 0.7
	Males	1039 ± 43.7	67.5 ± 3.0	15.4 ± 0.6
	t	11.4	4.4	6.5
	p	< 0.001	< 0.001	< 0.001
Vestigial:	Females	214 ± 221.8	8.7 ± 6.6	20.1 ± 11.3
TR both	Males	288 ± 245.6	12.0 ± 10.4	22.2 ± 11.9
wings	t	1.19	1.55	0.67
	p	> 0.10	> 0.10	> 0.10
Vestigial:	Females	103 ± 53.9	9.4 ± 3.8	10.8 ± 2.5
TR one	Males	101 ± 92.7	5.4 ± 2.6	16.7 ± 9.5
wing only	t	0.05	2.56	1.79
	p	< 0.10	< 0.05 > 0.02	> 0.10