embryo cells and L929 cells. Although the precise growth inhibition mechanism of the drug is yet unclear, the rate of RNA synthesis in the illudin S-treated cells markedly decreases without any effect on protein synthesis[9]. Inspite of such an antitumor effect, illudin S has not been taken up as an antitumor agent because of its effect. Our present series of experiments with illudin S has shown that enclosure of this compound into liposomes markedly enhanced its effect on survival time in Ehrlich ascites tumor, apparently by decreasing the side effects without diminishing its antitumor effect. As for the action mechanism of this increased antitumor effect by liposome entrapment, it was considered, as was reported for the action of actinomycin-D[10] and 1-β-D-arabinofuranocylcytosine[11], that the slow liberation of illudin S from liposome alleviated tissue damage and that increased contact between liposomes and tumor cells increased the affinity of illudin S to tumor cells, thus potentiating the antitumor effect of illudin S further.

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Estrogen receptor in uteri of mice of different *H-2* genotypes[1]

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Summary. A relationship between the amount of available estradiol receptors in uteri of inbred mice and their *H-2* genotype is suggested by study in congenic animals.

The *H-2* gene complex contains the major histocompatibility system of the mouse as well as genes which control various physiological processes e.g., immune responses to autoantigens[2], the level of cyclic AMP in the liver[3], the levels of serum hemolytic complement[4], testosterone and testosterone binding capacity[5], the 'mating preference'[6], and the level of fetal palatal glucocorticoid receptor[7]. In target tissues, estrogens bind to cytoplasmic receptors of high affinity and specificity; the steroid-receptor complexes are then taken up by the cell nucleus where the physiological effects of estrogens are initiated. We investigated whether the amount of estrogen receptors in the uteri of mature, virgin mice is genetically controlled and related to the *H-2* locus.

Materials and methods. Female mice, 8 weeks old, of different inbred strains, were obtained from Jackson Laboratories, Bar Harbor, Maine. Groups of 10–15 animals were housed in the same cage in air-conditioned quarters with 12-h lighting cycle and maintained for a week on a dried pellet diet and tap water ad libitum. A group of random bred male Swiss mice were kept in a separate cage in close proximity to the female mice. Vaginal smears and visual inspection of the vaginas[8] were used to determine the estrus cycle of the mice. Animals in the diestrous phase were killed by cervical dislocation and the uteri were quickly dissected out. Both uterine horns, from just below the entry of the oviduct to above the cervical junction, were separated from the remains of the uterus and used in the experiments. Because of the small size of the mouse uterus, 10–15 uteri from mice of the same strain were pooled. They were snapped frozen in liquid nitrogen or a mixture of acetone and dry ice, and stored in liquid nitrogen or at −85 °C. The estradiol receptor was assayed by a standard charcoal technique[9]. Briefly, the tissue was homogenized in ice cold Tris buffer, pH 7.4, with a steel mechanical homogenizer (Tekmar Company, Cincinnati, Ohio) and centrifuged for 30 min at 105,000 × g (Spinco model L5-40 ultracentrifuge, Beckman Instruments, Palo Alto, Cal.). The protein was determined by the method of Lowry et al.[10]. Increasing amounts of [2,4,6,7(n)-^{3}H]-estradiol (Amersham Corp., England) were added and plots were constructed from 6 points, with the bound estradiol, as described by Scatchard[11]. From these plots the dissociation constants (K_d) for the binding reactions were obtained. The estradiol receptor was expressed in fmoles/mg of cytosol protein. The analysis of the slopes of the Scatchard plots showed that the test was reliable with respect to variability within the assay and between assays.

Results. Table 1 shows a wide range of values for estrogen receptor in various inbred strains of mice. Some strains had

Table 1. Estrogen receptor in uteri of inbred mice

Strain	*H-2*	Estrogen receptor*	K_d**
C57BL/6J	b	68	1.2
C57BL/10J	b	27	1.3
LP/J	b	147	2.4
BALB/cJ	d	694	1.2
DBA/2J	d	358	2.4
AKR/J	k	774	1.2
CBA/J	k	661	1.0
C3H/HeJ	k	180	1.0
C3H/StJ	k	138	1.2
129/J	k	443	0.5
RF/J	k	339	1.2
DBA/1J	q	953	1.4
PUB/J	q	124	0.8
SWR/J	q	385	1.7
A.SW/J	s	430	0.6
SJL/J	s	684	0.6
SM/J	v	92	1.6

* ^{3}H-estradiol bound, expressed in fmoles/mg cytosol protein (pool of 10–15 uteri). ** Equilibrium dissociation constant (moles/l × 10^{-10}).

Table 2. Estrogen receptor in uteri of congenic mice

Strain	*H-2*	Estrogen receptor*	K_d**
C3H/HeJ	k	142	0.8
C3H.NB/J	p	161	1.9
C3H.SW/J	b	120	0.8
B10.D2/J	d	69	1.6
B10.Br/J	k	171	0.6
C57BL/10J	b	27	1.3

* ^{3}H-estradiol bound, expressed in fmoles/mg cytosol protein (pool of 10–15 uteri). ** Equilibrium dissociation constant (moles/l $\times 10^{-10}$).

relatively low estrogen receptor values (e.g., C57BL/6J, C57BL/10J, and SM/J) and some had high values (e.g., SJL/J, AKR/J, and DBA/1J) however, there was no clear-cut correlation with the *H-2* genotype. The values of the estradiol receptor in several congenic strains, i.e., which differ from each other only at the *H-2* locus but otherwise have the same genetic background, suggested an association with *H-2* since B10.D2, B10.Br, and C57BL/10J mice, which all have the same genetic background, had different levels of estradiol receptor (table 2). The K_d was in the same range of 10^{-10} moles/l for all the strains investigated.

Discussion. The total level of cytosol protein binding ^{3}H-cortisol in the palate was found to correlate with the *H-2* genotype, hence a product of a gene near or in the *H-2* locus seemed to be the glucocorticoid receptor[7]. However, the *H-2* was not the sole determinant of ^{3}H-cortisol receptor level because C3H/HeJ and CBA/J mice, both of *H-2*^{k} genotype, had different receptor levels. The findings presented here suggested an influence of *H-2* locus on the level of available binding sites for estradiol in mouse uteri. We did not measure the level of estradiol in the circulation of various strains of mice. If this level is influenced by the *H-2* locus similar to the influence on the testosterone level[5], then its possible influence on the uterine estrogen receptor could be less evident in our assay on intact animals. The individual values of estrogen receptors for each animal within the same strain were assumed to be similar; therefore uteri from 10–15 mice were pooled. The estrous cycle can influence the estrogen binding in uterus of rats[12]. However, there are controversial reports on the concentration of estrogen receptor in the uteri of rats in different periods of the cycle: increase in proestrus[13], decrease in proestrus[14] or no significant changes in the level of estrogen receptor throughout the cycle[15]. It is known that pheromones, excreted by males, cause synchronous estrus in mice[16], therefore we kept the female mice in the proximity of males for a week before the experiments.

Our results suggest an influence of the *H-2* locus on the amount of estrogen receptor in the mouse uterus. Genetic control of various proteins without a linkage with the major histocompatibility locus is well-known. For example, a genetic variation in male mouse kidney β-glucuronidase activity, not linked to the *H-2* locus, was described[17]. Further studies of mice in different periods of the estrous cycle as well as studies in individual inbred rats should shed more light on the possible genetic control of the uterine estrogen receptors.

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Production and characterization of antibody against aflatoxin M_1

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Summary. Antibody against aflatoxin M_1 was obtained after immunization of rabbits with bovine serum albumin-afla M_1 oxime conjugate. The antibody has greatest binding efficiency for afla M_1, and was less efficient for afla B_1. Cross-reaction of antibody with aflatoxin Q_1, aflatoxicol, and aflatoxin B_{2a} was weak. Aflatoxin B_2, G_1, and G_2 and afla B_1-guanine adduct showed almost no cross-reaction with the antibody. The sensitivity of the binding assay for aflatoxin M_1 detection is in the range of 1–10 ng per assay. Detailed methods for the preparation of the conjugate, production of immune serum, and methods for antibody determination are described.

Aflatoxin M_1 (afla M_1) is one of the major aflatoxin B_1 (afla B_1) metabolites in mammalian systems and has been shown in cow's milk, animal serum and urine[3–5]. It is also produced by several *Aspergillus flavus* isolates but in relatively small quantities in comparison with other aflatoxins[6]. In view of the occurrence of afla M_1 in milk due to ingestion of afla B_1-contaminated feed by cattle and its carcinogenic property, afla M_1 presents a potential hazard to human health[7–9]. It is essential to have simple, sensitive and reliable methods for monitoring afla M_1 in milk as well as other dairy products. Currently, both thin layer chromatography (TLC) and high pressure liquid chromatography