calculate the change in hole radius resulting from one stearate layer. Averaged over the three gases, this value, $(R_{\alpha 0} - R_{\alpha 1})_{av}$, is approximately 26 Å.

This same membrane was cleaned with ether to dissolve the stearate and was then rinsed successively with warm, concentrated KOH solution; water; concentrated chromic acid; water; and, finally, ethyl alcohol. The membrane was then etched further to increase the hole radius $(R_{\alpha' 0})$ to 140 Å (as determined by comparing Knudsen rates with the original 115 Å radius). A single layer on this enlarged radius gave a thickness $(R_{\alpha' 0} - R_{\alpha' 1})_{av}$ of 25 Å. The single layer was removed, and three layers were deposited, giving an equivalent thickness for the three layers $(R_{\alpha' 0} - R_{\alpha' 3})$ of 74 Å. Intermediate runs with the bare mica showed that within the precision of our experiments the cleaning procedure did not alter the hole size.

The next series of runs, designated by subscript β, refer to a hole of larger radius, approximately 240 Å. Here, as with the α membrane, one and then three stearate layers were deposited. The equivalent thicknesses were 27 and 81 Å. The last data, designated by subscript γ, are measurements made on a single membrane for monolayers of stearic, arachidic, and behenic acids (the fatty acids of chain length 18, 20, and 22 carbon atoms). These layers reduced the hole radius by 28, 31, and 35 Å, respectively.

The thicknesses of the layers calculated from the permeation rates show rough agreement with thicknesses of multilayers calculated for fatty acid soap films oriented with the fatty acid chain perpendicular to the solid surface. A typical value for the thickness of a calcium stearate layer as determined from x-ray diffraction measurements is approximately 25 Å (16). The fact that the triple layers display a thickness three times that of the monolayer suggests that the perpendicular orientation is maintained in the multilayers. Also, the increment in thickness for the arachidate and behenate layers is of the order one would expect for the increased chain length.

In the preceding discussion, the pores in the mica have been considered to be circular. Actually, they are rhombi with angles of 60° and 120°, resulting from the basic crystalline structure of mica (17). Treating the rhombi as equivalent circles results in little error

as far as the Knudsen determinations are concerned because the Knudsen conductance for a tube of uniform cross section depends on the ratio of the square of the cross section to the perimeter (18), a factor which differs by less than 2 percent for a 60° rhombus and the equivalent circle. That is, the circle and the rhombus of equivalent conductance have side and diameter which differ by less than 2 percent.

In certain respects the thicknesses of the layers are more uniform than one would expect, especially those of the tri-layers. Obviously, with the smaller inner perimeter of each additional layer, the head-to-head and tail-to-tail spacing cannot be the same for each layer. Also, the layer spacing in the corners of the rhombi—where the radii of curvature are greatest—cannot be completely regular. The precision of our present determinations is reflected in the range of thicknesses, 25 to 28 Å, measured for a single stearate layer. The scatter of the data appears to be random, with equivalent results for the small diameter and the large diameter pores.

Although our measurements have been restricted to the permeation of gases, there is no reason to expect that these membranes would not operate successfully in an aqueous environment. Also, the wall coatings are not limited to fatty acids.

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Thymus and Reproduction : Sex-Linked Dysgenesia of the Gonad after Neonatal Thymectomy in Mice

Abstract. *Neonatal thymectomy of mice, when no ectopic thymus existed, constantly resulted in developmental arrest of the ovary but not of the testis; it also caused sterility in the female. The ovaries of thymectomized mice were extremely small and were characterized by absence of follicles and corpora lutea. Such an ovarian dysgenesia was observed when the mice were thymectomized at 3 days of age, but not at 7 days or later; it was prevented by thymus grafting.*

The essential role of the thymus in immunology has been established in many species of animals (1). Although the thymus has long been considered to be an endocrine organ somehow related to sexual physiology, no evidence has yet been presented. Thymectomy at 3 days of age in C3H/HeMs and (C3H/

HeMs × 129/J)F$_1$ mice apparently results in a reduced frequency of mammary cancers and retarded development of mammary glands (2). This suggests that thymectomized mice may be in a hormonal imbalance by which mammogenesis is probably controlled. If so, it is possible to postulate that the thymus has an undescribed function related to reproductive physiology. We now report experiments which point out that dysgenesia of the ovary can be induced by complete removal of the thymus at a critical age after birth.

Female and male mice of (C3H/HeMs × 129/J)F$_1$ and (C57B1/6J × A/Jax)F$_1$ were used. The thymus was removed at 3 days of age as described (2). Alternate littermates were used as sham-thymectomized controls; that is, they underwent the full operative procedure, but their thymus glands were left intact. Females and males were segregated at the weaning age. No antibiotics were administered, and no further treatment was given after surgery. Thymectomy at this age did not result in any wasting syndrome (2). There were no differences in mortality after the operation, weight curves, or susceptibility to common laboratory infections between the thymectomized and sham-thymectomized groups in the hybrid combinations used.

Groups of mice of both sexes, thymectomized and sham-thymectomized, were killed either at 30 days of age or thereafter at intervals of 30 days up to the age of 180 days. The completeness of thymus removal was verified at autopsy. Weights of the spleen, liver, kidney, mesenteric lymph node, salivary gland, adrenal, and ovary and uterus or testis and seminal vesicle were measured on a torsion balance. The most significant result was found in changes of ovarian weights with age (Fig. 1). In mice that underwent sham-thymectomy, ovarian weight increased progressively in the first 120 days of life, reaching the normal adult weight of 8 to 18 mg. The ovaries of thymectomized mice were on gross inspection normal until the animals reached the age of 60 days; at 90 days and thereafter the ovaries of more than 50 percent of the mice showed comprehensive developmental arrest. Some ovaries were extremely atrophic. The same ovarian changes occurred in both (C3H × 129)F$_1$ and (C57 × A)F$_1$ mice.

In an attempt to investigate the relation of thymectomy at varying ages to the inhibition of the ovarian development, we removed the thymus gland when the mice were 7, 20, and about 40 days of age (the age when first estrous vaginal smear is positive). The result indicated that thymectomy after the age of 7 days was no longer associated with gonadal development, an indication of a critical age at which thymectomy induces ovarian dysgenesia.

Histologic examination revealed that the small-sized ovaries of thymectomized, 90- to 180-day-old mice showed, without any exception, conspicuous decrease or complete disappearance of ripening follicles of all sizes and absence of corpora lutea; the examination also revealed the degenerative follicles in some ovaries of thymectomized, 60- to 90-day-old mice. The parenchyma of these atrophic ovaries was almost entirely occupied by the interstitial gland cells, which were not atrophic but rather hypertrophic (Fig. 2). This characteristic histology is comparable to that of ovaries after irradiation (3), and the picture is in striking contrast to the histology of atrophic ovaries of hypophysectomized mice in which numerous small-sized follicles of normal appearance are preserved (4). It seems likely, therefore, that thymectomy at 3 days of age leads to an injury of very early ovarian follicles, in contrast to hypophysectomy, after which the ovary shows normal follicles.

No abnormality in testicular weights or histology was noticed during the 180 days of observation after thymectomy at 3 and 40 days of age. No
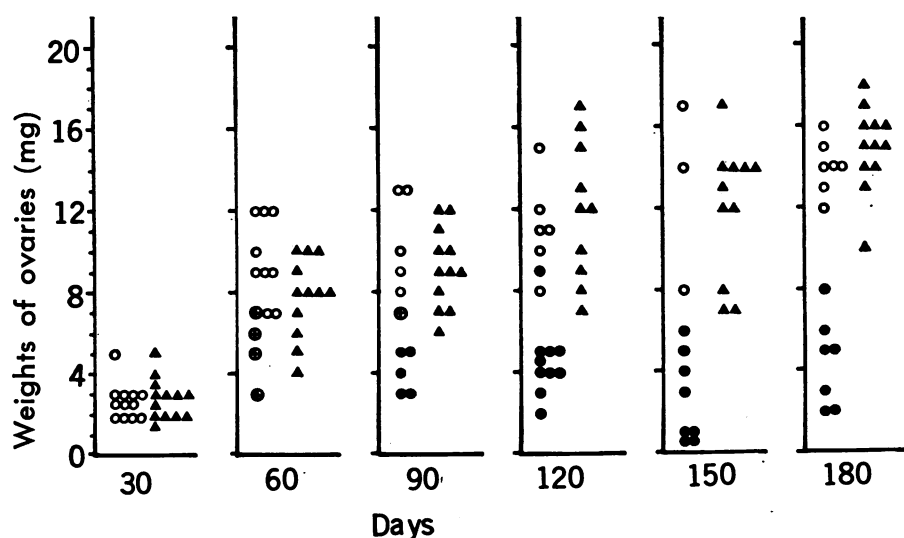
Fig. 1. Weight changes in ovaries (at day indicated) of (C3H/HeMs × 129/J)F$_1$ mice thymectomized at 3 days of age. Each symbol indicates total weight of both ovaries in each animal. Open circles, thymectomized mice with ovaries of normal histology (121 ± 48.2 mg, average weight ± S.D., of uteri of mice killed at 120 to 180 days of age); solid circles, thymectomized mice with atrophic ovaries as illustrated in Fig. 2 (70.3 ± 35.7 mg); hatched circles, thymectomized mice with ovaries containing many degenerative follicles; solid triangles, sham-thymectomized mice with normal ovaries (120.9 ± 55.6 mg). Of 19 mice thymectomized within 24 hours after birth, 13 died of the wasting syndrome within 120 days after surgery; all the survivors at 120 days of age had normal ovaries and ectopic thymus in the thyroid tissue.
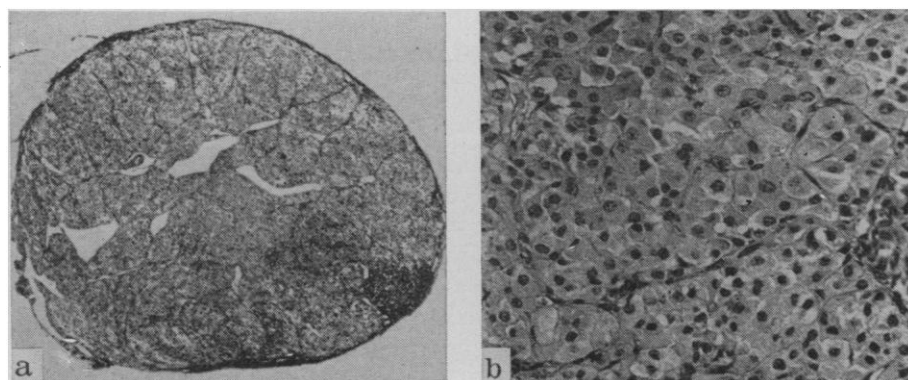
Fig. 2. Atrophic ovary of a mouse thymectomized at 3 days of age and killed at 120 days. Weights of ovaries and uterus are 5.0 mg and 82 mg, respectively [hematoxylin and eosin; (a) × 40; (b) × 240].
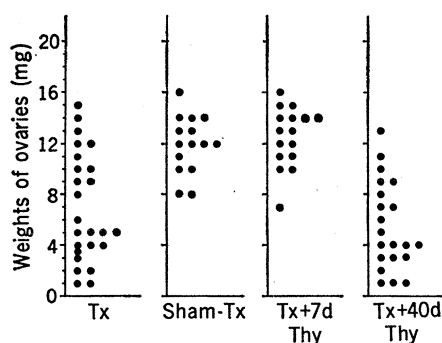
Fig. 3. Recovery of ovarian atrophy by thymus grafting in (C3H/HeMs × 129/ J)F₁ mice; *Tx*, thymectomized at 3 days of age; *Sham-Tx*, sham-thymectomized at 3 days of age; *Tx+7dThy*, thymectomized at 3 days of age and grafted with newborn thymus at 7 days; *Tx+40dThy*, thymectomized at 3 days of age and grafted with newborn thymus at 40 days. All the mice were killed at 120 days of age. Each symbol indicates total weight of both ovaries in each animal.

change in the ovary could be recognized in the mice splenectomized at this age.

Examination on fertility of (C3H × 129)F₁ thymectomized mice showed that 36 out of 48 operated females (75 percent) were sterile, and the ovaries were constantly small and atrophic (at autopsy) at the age of 180 days. In these animals irregularity of estrous cycles—absence of pro-estrous and estrous stages—was seen on vaginal smears. In 20 males that were thymectomized at 3 days of age, no decreased capacity of fertility was found.

To account for the data that 25 to 40 percent of animals do not show the ovarian dysgenesis after thymectomy at 3 days of age, we searched for ectopic thymus outside the mediastinum; ectopy was frequently detected within the thyroid of BALB/c mice (5). Thirty-five female (BALB/c × 129/J)F₁ mice were thymectomized, as described above, and killed at the age of 360 days. Fifteen mice (43 percent) had normal ovaries (> 8 mg), and the remaining 20 had atrophic ovaries (< 7 mg). Serial sections of the thyroid were made on 22 mice, randomly selected, and thymic tissue was found in the parathyroid area in ten mice whose ovaries were normal in size (> 8 mg) and histology. The remaining 12 mice with atropic ovaries (< 5 mg) had no ectopic thymus. This was also true for the (C3H × 129)F₁ mice thymectomized at 3 days of age: eight mice with normal ovaries had ectopic thymus and ten with atrophic ovaries had no ectopic thymus.

The next experiments were concerned with the effect of thymus replacement (Fig. 3). Mice of (C3H × 129)F₁ and (129/J × C57B1/6J)F₁ hybrids received thymectomy at 3 days of age which was followed by grafting at 7 days of age with one whole intact thymus from 1- or 7-day-old female or male donors into the No. 4 fat pad. All these mice had ovaries of normal size and morphology at subsequent autopsy (120 days of age). On the contrary, no recovery of the ovarian changes was attained in the thymectomized mice similarly grafted with thymus at the age of 40 days. These data indicate that the thymus grafts, only if implanted shortly after the thymus removal, can prevent ovarian dysgenesis.

Correlation between the gonad dysgenesia and immunologic function after thymectomy at 3 days of age was investigated. In both males and females of (C3H × 129)F₁ hybrid tested at the ages of 120 and 180 days, the thymectomy resulted in a moderate lowering of circulating lymphocytes, considerable decrease in number of plaque-forming cells in the spleen as determined by the technique of Jerne and Nordin (6), and a slight, borderline prolongation of the survival of allogenic skin grafts. No significant difference in these immunologic responses could be found between the thymectomized mice with normal ovaries and those with atrophic ovaries. Therefore, there is still no positive evidence that sex-linked developmental failure of the gonad is essentially related to depressed immunologic faculty after thymectomy at 3 days of age.

In view of our results, it seems plausible to propose that the thymus has the newly described function of controlling the reproductive faculty in the female mouse. However, we should consider the possibility that viral infection—mostly affecting the ovary after the thymectomy—may be a cause of the ovarian changes.

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Berberine: Complex with DNA

Abstract. A complex of calf-thymus DNA with berberine sediments in the analytical ultracentrifuge. The DNA produced systematic changes in the absorption spectrum of berberine which suggest that single alkaloid molecules bind to DNA. Flow dichroism of purines and pyrimidines and of berberine in the complex with DNA had the same signs and magnitudes. Berberine shifted the thermal strand separation profile of DNA to higher temperatures. Therefore, the alkaloid forms a complex with DNA, probably by intercalation.

Among substances which form complexes with DNA are basic dyes (1), numerous antibiotics (2), and synthetic drugs (3). We have shown that a medically important alkaloid, quinine, forms a complex with DNA (4) and report here studies on a second example of interaction of an antimicrobial alkaloid, berberine, with DNA.

Berberine is widely distributed in higher plants (5). The alkaloid has antibacterial (6) and antiprotozoal (7) activity. By optical methods, berberine has been shown to interact with nucleic acids (8, 9), especially DNA (10). Like acridines (11) or ethidium bromide (12), berberine converts yeast to respiratory-deficient cells which grow in "petite" colonies (13).

Table 1. Flow dichroism of the DNA-berberine complex. The method has been described previously (15, 16). Dichroism is expressed as fractional change in absorbancy when the complex was flow oriented. The DNA concentration was 2 mg/ml; berberine, $2 \times 10^{-4}M$; and tris(hydroxymethyl)aminomethane-HCl buffer, $5 \times 10^{-3}M$ at pH 7.5. The light path was 0.25 mm; 0°, plane of polarized light parallel to axis of flow; 90°, plane of polarized light perpendicular to axis of flow.

Dichroism	259 nm	350 nm
At 0°	− 0.22	− 0.18
At 90°	+ .09	+ .08