changes observed in the blood serum.

These measurements reflect changes in concentration of the serum proteins only. Since blood volume values were not obtained in this study, conclusions regarding changes in the total serum protein mass cannot be derived.

Moore(14) has found lower levels of Vit. A in the liver of animals on the Vit. E deficient diet. The integumentary changes in our group of experimental chicks may have been a manifestation of Vit. A deficiency rather than Vit. E deficiency.

Summary and conclusions. 1. Chicks fed on an experimental diet, high in linoleic acid and deficient in antioxidants, developed a progressive disease manifested by integumentary changes and signs of central nervous system impairment. 2. Blood serum protein patterns were altered and were characterized by an increase in total protein, a decrease in the A/G ratio, and an increase in beta and alpha 2 globulin fractions. 3. Despite a decreasing A/G ratio, chicks on the experimental diet did not develop exudative diathesis. 4. It appears that the blood serum protein changes are a manifestation of Vit. E deficiency.

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The Mammary Tumor Agent in Completely Mammectomized Mice.* (28933)

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Although the mammary tumor agent (milk factor, Bittner virus) has been studied for many years, our knowledge, especially of the biology of this agent, is scarce(1-3). Nothing is known about the tissues or organs in which this agent multiplies in the mouse. Its only known effect is the promotion of cancer in the mammary glands of mice. It would seem reasonable to assume that the mammary gland is the organ in which the agent develops and propagates. Lasfargues *et al*(4) conclude that the glandular stroma of the mammary gland is involved in the multiplication of this agent. It is known, however, that the tumor agent can propagate in male animals in which there only rudimentary mammary glands.

One of us (A.D.)(5) has developed a technique to extirpate all mammary glands from a mouse. Such mammectomized mice can be used to test the propagation of the

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mammary tumor agent in the absence of the mammary glands.

Young mice were mammectomized and injected with the mammary tumor agent. Because the spleen contains the agent, it can serve as a good indicator for the presence of the agent. Therefore, after 4 months the spleen was transplanted to young mice with the same genetic constitution. The mammary tumor incidence in these host mice was recorded and compared with control groups.

Materials and methods. (♀ C_3H_f × ♂ 020)F_1-female mice were used. These mice do not harbor the mammary tumor agent (3,6). Twenty-five females at the age of 19-21 days (group A) were mammectomized with the technique described earlier(5). The same number of females (group B) were unoperated and served as controls. The completeness of the mammectomy was determined by careful inspection of the skin at autopsy. Any bits of adipose tissue found in the region from which the mammary glands were removed and which might have contained the remnants of the mammary gland tissue were dissected from skin after fixation, stained with hematoxylin and examined under the dissecting microscope.

Within one week after the operation the mammectomized mice and the controls were injected with an extract from mammary tumors of the C_3H-strain containing the mammary tumor agent. For preparation of the extracts, the tumors were ground with sand, and distilled water was added (5 ml to 1 g tumor tissue). After centrifugation in an ordinary laboratory centrifuge 0.1 ml of the supernatant was injected intraperitoneally.

All mice of both groups were then forcebred for 3 months; 2 females were kept in a cage with one male and the young were discarded immediately after birth. At the age of 5-6 months the spleen was removed surgically from each animal of the test and the control group. The spleen was divided into 6 fragments and 2 fragments were transplanted subcutaneously into each of 3 (♀ C_3H_f × ♂ 020)F_1-virgin females 3-weeks old. The group receiving spleen from group A was called group C, and the animals receiving spleen from group B designated group D.

The animals were kept as virgins, 3 in a box. Observations were made at weekly intervals, the mammary tumors being noted as appearing on the date when first palpable. Histologic diagnosis was made in all cases. The tissues were fixed in Susa solution and stained with hematoxylin and axophloxin.

Results. Group A. Mammectomized (♀ C_3H_f × ♂ 020)F_1 hybrids treated with mammary tumor agent. From the group of 25 females, 2 later showed incomplete mammectomy. In one of these 2 mice a mammary tumor developed; the other one died accidentally at an early age. These 2 animals have been discarded. None of the other 23 females has developed mammary carcinoma, although 20 of these animals lived longer than 2 years after termination of the experiments. This absence of mammary carcinoma at this age proves the completeness of mammectomy.

Group B. Twenty-four of 25 normal (♀ C_3H_f × ♂ 020)F_1 hybrids treated with mammary tumor agent developed mammary carcinomas at the average age of 351 days. The injected extract obviously contained the mammary tumor agent.

Group C mice with transplanted splenic tissue from mammectomized donors and *Group D* mice with transplanted spleen from normal donors had comparable and high mammary tumor incidences (Table I).

Discussion. As to whether the mammary tumor agent can propagate or persist in a mouse without mammary glands, the results in group C and D are decisive. Mammary tumors appeared in previously agent-free mice implanted with splenic tissue from donors without mammary glands. That no mammary tumors developed in the virgin test animals without the mammary tumor agent means that the agent was transferred with the transplanted spleens from the mammectomized mice. The agent persisted and/or propagated in a mouse in the absence of mammary glands. It is known that a quantitative relation exists between amount of agent given and time of appearance and incidence of mammary cancer(2). One would expect

TABLE I. Mammary Tumor Incidence in (♀ C_3H_f × ♂ 020)F_1 Hybrids with Transplanted Spleens from Mammectomized and Normal Donors.

	No. of animals	With mammary tumors	Avg tumor age (days)	Avg age at death without tumors (days)
Group C With spleen from mammectomized donors	66	60 = 91%	322	549
Group D With spleen from normal donors	73	70 = 96%	321	561
Untreated controls	50	0%	—	728

that the developed mammary glands in several pregnancies would favor propagation of the agent. The results in Table I show, however, that mammary tumor incidence and especially average tumor age in both Groups C and D are exactly the same.

Essential for evaluation of these results is the completeness of the mammectomy. At autopsy, all mice were checked for the rests of the mammary glands. It is unlikely that even tiny rests of the mammary glands could not have been found. All the mice have been force-bred. Small bits of the mammary glands can grow to normal sized glands(7). Another indication of completeness of mammectomy is the absence of mammary tumors in mice harboring the tumor agent. In incompletely mammectomized mice, mammary tumors develop in a high percentage (Fekete, 8) indicating that mammary tumors may develop in small rests of the glands. In another as yet unpublished investigation with 200 mammectomized mice 10 (5%) were incompletely operated. In 8 out of these 10 mice, mammary tumors developed at a slightly higher age than in the unoperated controls. The other 2 died before reaching the tumor age.

These results give no support to the hypothesis that propagation of the tumor agent is restricted to the mammary glands or favored by their presence. From the results of these experiments the site of propagation of the mammary tumor agent could not be determined.

Summary. Mammectomized female mice of the mammary tumor susceptible hybrid (♀ C_3H_f × ♂ 020) group retained or propagated the mammary tumor agent. Two groups of agent-free recipients of splenic tissue from either mammectomized or intact donors that had been previously exposed to mammary tumor agent had a high incidence of mammary cancers at comparable ages. Controls not receiving splenic tissue failed to show mammary tumors.

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