That the development of mammary cancer in mice is dependent, in part, upon the hormonal stimulation of the mammary glands has been acknowledged for many years (27). It had also been assumed that ovariectomy of young females of cancerous stocks would greatly reduce the incidence of mammary cancer, until, in 1939, Woolley, Fekete, and Little (17, 86) reported that excision of the ovaries from 1-day-old mice of the dilute brown stock had little, if any, effect in altering the incidence of mammary cancer, although the time of development of the tumors might have been delayed.

These investigators noticed nodular hyperplasia of the adrenal cortex in the ovariectomized females, and, since there was hormonal stimulation of the uterus and development of the mammary glands, they concluded that the adrenals had simulated the function of the ovaries.

The same histological type of adrenal response was seen in ovariectomized females of the cancerous C3H strain (37), whereas mice of the C57BL (37) and the A (16) strains remained in the castrate state.

To determine if mice of various strains are susceptible to spontaneous mammary cancer, females should be observed which have an adequate hormonal stimulation, such as that which results from breeding, and which also possess the mammary tumor agent (5–8), for the incidence of mammary cancer in nonsusceptible animals will be found to be approximately the same as in susceptible mice of most stocks which do not possess the mammary tumor agent.

After females of some sublines of the C57BL stock have been nursed by females of cancerous strains, the incidence of mammary cancer in breeding females may be from 63 to 76 per cent (2, 4, 22). This incidence is approximately the same as that observed in some so-called susceptible mice with the mammary tumor agent (8). When C57 mice with the agent were continued as nonbreeders, 9 and 13 per cent gave rise to mammary tumors (28, 35). Likewise, virgin females of the A strain showed a low incidence, while a high percentage of the breeders developed these tumors (5, 13).

Postcastrational hyperplastic adrenals were found (36–38) in females of strains which showed a high incidence of mammary cancer in virgin females (1, 18, 28, 29, 35), while those strains which showed few tumors in nonbreeders remained in the castrate state (5, 13, 16, 28, 29, 35, 37).

Thus, these observations obtained on ovariectomized females of various stocks suggested a possible association between the adrenal response and the development of, or the ability to induce, mammary cancer in virgin or nonbreeding females of strains which are susceptible to mammary cancer and possess the mammary tumor agent.

Since 1944, numerous reports from this laboratory have been concerned with the development of mammary cancer in virgin females of inbred stocks and their hybrids, associated with various inherited hormonal patterns or mechanisms, as indicated by postcastrational adrenal cortical alterations. In these studies, females of the A strain, with a low incidence in virgins, were crossed with males of stocks showing the adrenal lesions, and the resulting hybrids were continued as either virgins or breeders. The mothers of the A strain would contribute the mammary tumor agent and the inherited susceptibility for mammary cancer; the paternal parents would transmit an inherited hormonal mechanism and, in certain cases, they were also susceptible to mammary cancer.

In the original cross, females of the A stock were mated with males of the Z (C3H) cancerous strain, and the F1 virgin females were found to have a...
high incidence of mammary cancer, demonstrating that the hormonal pattern which determined a high incidence in the C3H virgin females was transmitted (15, 23). This mechanism has been termed the inherited hormonal influence for mammary cancer (7).

Investigation of an inbred stock in which high incidences of mammary cancer were observed in virgins having the inherited hormonal influence was undertaken to ascertain whether postcastrational adrenal cortical hyperplasia would develop in gonadectomized females and hybrids (7, 15). Females of the A and Z (C3H) strains and their hybrids were ovariectomized. While the castrate females of the A stock showed only slight adrenal changes, there was little or no evidence of hormonal production, as determined by the castrate state of the accessory sex organs (80—83), confirming the observations of Woolley (84). The majority of the Z mice and their hybrids had adrenal lesions with hormonal stimulation of the uterus and mammary glands. Although the castrated mice of the Z stock and their hybrids without the mammary tumor agent showed the adrenal abnormalities, mammary tumors were found only in the females of these groups which possessed the agent. Thus, the tendency for postcastrational adrenal lesions to develop, characteristic of the Z stock, was found to be transmitted as a dominant over the absence of this condition. Also, in these stocks and their hybrids, it was associated with the inherited mammary cancer-inducing hormonal influence (7) for virgin females.

By extending these observations and testing various strains which develop postcastrational adrenal changes, several have been found to have the inherited hormonal influence which induces mammary cancer in virgin females, either in inbred stocks or their hybrids (8—11, 14, 24). Also, by using females of the Z stock as mothers of other hybrids, possible differences between inherited hormonal patterns may be seen as they influence the genesis of mammary cancer in mice.

In 1941, Gardner (82) ovariectomized females of the Strong NH stock (35) and found that thirteen of fifteen animals later developed adrenal tumors and two had mammary cancer. From the condition of the bony pelves and the secondary sex organs, it seemed probable that the adrenal lesions produced estrogen primarily. Confirmatory data were reported by Kirschbaum and his associates (19, 20, 25, 26) for mice of the same stock. In addition (20), spontaneous adrenal tumors were found in intact NH females at 1 year of age, but the development of lesions could be accelerated by removing the ovaries from young mice. Estrogen was shown to be the primary secretion from the altered adrenals.

Because of these observations, mice of the NH stock were tested for the transmission of the inherited hormonal influence which would be expected to produce a high incidence of mammary cancer in ANHF1, virgins (A 9 X NH 9). ZNHF1 (Z 9 X NH 9) were observed also.

MATERIALS AND METHODS

The three inbred strains used in these experiments were developed by Strong (35, 34). The A and C3H (Z) stocks were separated from Strong’s lines at least 20 years ago, while representatives of the NH strain were obtained from Kirschbaum in 1946. As in previous reports, to simplify the designation of the hybrid generations, the C3H will be referred to as the Z stock. All strains have been maintained by brother-to-sister matings.

In Table 1, information is given on how the various hybrids were produced. When females of the A strain were crossed with NH males, the resulting hybrids were called ANHF1. The females of the ANHF1-AxBC and ANHF1-ZbBC generations were derived by crossing the F1 females with Ax or Zb males, respectively. These males were from lines which did not possess the mammary tumor agent (7).

The ZNHF1 (Z 9 X NH 9) females also were mated with either Ax or Zb males, and, for convenience, these hybrids have been called ZNHF1-AxBC or ZNHF1-ZbBC animals, depending upon the paternal parents.

All females received Purina Fox Chow and tap water. Details regarding housing are the same as used for mice of other crosses (8, 12).

EXPERIMENTAL RESULTS AND DISCUSSION

Observations are presented in Table 1 on the development of mammary cancer in virgin and breeding females on inbred stocks and their hybrids, except for mice of the NH stock.

Breeders of the NH stock remained free of mammary cancer when they did not possess the mammary tumor agent. The number living for 10 months, or longer, was 118. Many were not continued after they were removed from the breeding pen, at approximately 1 year of age, while others survived for nearly 2 years. The average age for the entire group was 15 months.

Only preliminary details may be given for NH females which were fostered by females of the cancerous Z stock and for the descendants of these NH mice. In a group of ten, three have had mammary cancer (average age, 271 days), three died...
without cancer, and four are living and are between 18 and 15 months of age. Other NH females have been fostered, but they are too young to be included.

Details regarding the other observations will be considered in the discussion.

In virgin female mice the development of mammary cancer has been found to be dependent upon an inherited hormonal mechanism (15, 23), now called the inherited hormonal influence (7), in addition to the inherited susceptibility which in certain stocks and their hybrids has been shown to be associated with postcastrational adrenal cortical hyperplasia (30–32), and the mammary tumor agent (5–8). By testing various strains, this mammary cancer inherited hormonal pattern has been found to be transmitted by, in addition to the Z, the dilute brown (sublines D2 and D3), the C and ANHF1 hybrids, whether they were maintained as virgins or breeders. However, when Zb males, known to transmit the inherited hormonal influence, were crossed with the ANHF1 females, higher incidences of mammary tumors were found in their progeny, in virgins as well as breeders; but the time of appearance of the tumors was not accelerated (Table 1).

The ANHF1-ZbBC (F1 9 X Ax) hybrids showed approximately the same results as did the ANHF1 hybrids, whether they were maintained as virgins or breeders. However, when Zb males, known to transmit the inherited hormonal influence, were crossed with the ANHF1 females, higher incidences of mammary tumors were found in their progeny, in virgins as well as breeders; but the time of appearance of the tumors was not accelerated (Table 1).

Females of the Z stock also were mated with NH males to give ZNHF1 hybrids, in order to study the hormonal patterns transmitted by the parental strains. At least 90 per cent of the ZNHF1 females developed mammary cancer, and a difference of 5 months was seen in the average ages of virgins and breeders (Table 1). Approximately the same incidence noted in breeders of the A stock was found in the hybrids resulting from the cross between the ZNHF1 females and Ax males, and the virgins of this group had a higher incidence than did either the ANHF1 or ANHF1-AxBC virgins. Probably, mammary tumors should have appeared in more of the ZNHF1-ZbBC hybrids, compared to the F1 population, but the time of appearance of their tumors was comparable to that of the mater-

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**TABLE 1**

<p>| TABLE 1 | MAMMARY CANCER IN INBRED MICE AND THEIR HYBRIDS |
|----------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>STOCK OR GENERATION</th>
<th>CROSSES</th>
<th>VIRGIN FEMALES</th>
<th>BREEDING FEMALES</th>
</tr>
</thead>
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<tr>
<td>ANHF1</td>
<td>A 9 XNH</td>
<td>185</td>
<td>16</td>
</tr>
<tr>
<td>ANHF1-AxBC</td>
<td>F1 9 XAx</td>
<td>50</td>
<td>14</td>
</tr>
<tr>
<td>ANHF1-ZbBC</td>
<td>F1 9 XZb</td>
<td>69</td>
<td>61</td>
</tr>
<tr>
<td>ZNHF1</td>
<td>Z 9 XNH</td>
<td>68</td>
<td>94</td>
</tr>
<tr>
<td>ZNHF1-AxBC</td>
<td>F1 9 XAx</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>ZNHF1-ZbBC</td>
<td>F1 9 XZb</td>
<td>51</td>
<td>68</td>
</tr>
<tr>
<td>NH</td>
<td>NH 9 XNH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See text.*
nal Z stock. In several groups, only a small number could be maintained as virgins, for several other crosses were being observed concurrently.

Hybrids of seven crosses have been observed where females of the Z stock have been mated with males of other strains. In virgins of these F1 groups, the lowest incidence and latest cancer age were found in the hybrids with fathers of the A strain, a stock with a low incidence of mammary cancer in virgins, whose breeders are susceptible to mammary cancer (5) and whose ovariectomized females remain in the castrate state (30—32). The earliest average cancer age, among both F1 virgins and breeders, and a high incidence occurred in the ZI cross, when males of a nonsusceptible strain were tested (1, 12). With the exception of the ZAF1 group, the ZNHF1 virgins had the latest tumor age, and, in the breeders, it was 1.5 months later than that observed for the ZAF1 breeders.

These data might indicate that the inherited hormonal pattern transmitted by mice of the NH strain could, at this time, be considered as distinct from that which has been called the mammary cancer-inducing inherited hormonal influence for virgin females (7). Instead of producing a high incidence of mammary cancer in virgin animals which are susceptible and have the mammary tumor agent, such as the ANHF1 hybrids, evidence was obtained in the ANHF1 and back-cross hybrids that the hormonal mechanism characteristic of the NH stock tends either to inhibit or to delay the time of development of these tumors, not only in virgin females but also in breeders. The inherited hormonal influence for mammary cancer in virgin females, as possessed by the Z stock, was found to be dominant over the hormonal mechanism transmitted by the NH mice, although some effect of the NH pattern may be apparent in influencing the average cancer age in the hybrids.

Before it will be possible to establish definitely the influence of various inherited hormonal patterns on the genesis of mammary cancer in mice, consideration must be given to the possibility that the other causative factors for mammary cancer may interact to produce the observed results. Previous reports (7—14), and others reviewed in (8), have shown that, when mice of various cancerous stocks were mated, the reciprocal hybrids may not show comparable incidences and average cancer ages. These data suggested that the mammary tumor agents transferred by mice of different cancerous stocks did not have the same tumor-inducing activity in reciprocal hybrids possessing the same genetic constitution for the development of spontaneous mammary cancer. In some crosses, the percentage of the F1 virgins, derived by crossing two cancerous stocks, to have mammary cancer was only slightly higher than that observed in the ANHF1 virgin females. However, while the ANHF1 breeders continued to have a comparatively low incidence (Table 1), high incidences were observed in the F1 breeders of the other series ([8] and unpublished data).

Some influence of a possible difference in the activity of the mammary tumor agent in the hybrids of the NH mice may be apparent in the following data, although the numbers are small and some animals are still under observation.

A few of the first and second generation descendants of the fostered NH females, which obtained the mammary tumor agent by nursing females of the cancerous Z stock, were mated with Zb males. Preliminary data may be cited on the development of mammary cancer in their F1 hybrids, which were maintained as breeders to study the propagation and transmission of the agent in NH mice. No difference has been found in the cancer incidence in the hybrids, which can be related to the generation of the NH mothers, and the youngest living animals are 9 months of age. In depleted litters, thirteen (100 per cent) have had mammary tumors at an average age of 293 days. In undepleted litters, fifteen have had tumors (average age, 276 days), ten are living, and one succumbed without cancer at 330 days of age. Thus, of these F1 females with mothers of the NH stock with the agent, 28 of 29 (97 per cent) which have died to date have had mammary cancer. While the results may be altered after all the animals have died, there is considerable difference, even in depleted litters, between the average cancer age and that seen in the ZNHF1 breeders (Table 1).

Thus, the activity of the mammary tumor agent must be given some consideration in the interpretation of data on the genesis of mammary cancer in mice, for the agent transferred by females of a cancerous strain may have different tumor-inducing properties when it is propagated and transmitted by animals of a different genetic constitution.

**SUMMARY**

A small number of fostered NH females with the mammary tumor agent were observed, and a few developed spontaneous mammary cancer.

Since mice of the NH strain develop spontaneous and postcastrational adrenal cortical alterations, they were tested to determine if they might transmit the mammary cancer inherited hormonal influence, which induces mammary cancer in virgin females that are susceptible and have the mammary tumor agent.

When females of the A strain, with a low incidence in virgin females, were mated with NH
mammals, the development of mammary cancer in the ANHF₁ hybrids, even in breeders, was either delayed or inhibited, possibly because of the action of the NH hormonal mechanism.

By mating NH males with females of the Z stock, known to transmit the mammary cancer inherited hormonal influence, a high incidence of mammary cancer was observed in the Z/NHF₁ virgin females.

By mating the ANHF₁ females with males of the Z stock, an increased incidence was seen in the progeny when they were kept as nonbreeders. Other observations were reported upon the development of mammary cancer in back-cross animals of different genetic constitutions.

While the data suggested that the hormonal pattern transmitted by the NH mice may have an inhibitory effect on the development of mammary cancer, consideration must be given to the interaction of the other causative factors, together with possibly different inherited hormonal mechanisms.

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Inherited Hormonal Mechanisms and Mammary Cancer In NH Mice and Their Hybrids

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