Spontaneous Gastric Neoplasia in Mice of the Br-S Strain
Incidence and Genetic Linkage Tests*

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INTRODUCTION

Several differing strains of mice have been produced from the NHO stock (7) as a result of sib-inbreeding associated with repeated subcutaneous injections of methylcholanthrene. One of these strains, Br-S, is characterized by a high incidence of gastric adenomas or adenocarcinomas. The evidence for a hereditary basis of the abnormality is extensive (7—10). However, the etiology is not clear; studies of the gastric pathology (2—4, 6, 11) in this strain indicate considerable variability, and the term "lesion" has often been used instead of tumor. Andervont (1) also uses the term "lesion" for the adenomatous condition characteristic of the I strain. The latter is not the same as that of the Br-S strain in two respects: (a) position of origin—at the forestomach junction in strain I mice, but near the pylorus in Br-S; and (b) inheritance—outcross F1 hybrids have the lesion in the case of Br-S, but not in the case of I. The two strains have not yet been crossed with one another.

It is the purpose of the present paper to give data on the incidence of gastric neoplasia in an untreated sub-line of the Br-S and a further study of the genetic basis of the condition in an outcross. The term "tumor" will be used, without pathological certainty, but simply in preference to the more ambiguous "lesion." Other kinds of tumors (e.g., lung tumors) and pathological conditions will be omitted from consideration, since they were infrequent and seem to bear little if any relationship to the main problem.

MATERIALS AND METHODS

The untreated sub-line of the Br-S strain was started in 1944 with ♀190592 and her two brothers, in the F12 generation (see Strong, 1945 [7], Fig. 1, "G and F"). The female and one brother developed tumors of rather large size (autopsy at 1½ years old or more). The other male died before 1 year old, and no necropsy was obtained. Brother-sister matings have been continued in all generations. Residual genetic segregation may have differentiated slightly differing sub-lines within this descent, but all the descendants of the above three mice are here grouped together. Selection has not been consciously practiced.

The care of the mice is the same as described earlier (7). Some of the mice were arbitrarily chosen as breeders, while others (a greater number) were segregated as to sex ("nonbreeders"). The breeders were permitted to raise from one to four litters of young, and after that their young were discarded soon after birth. The breeders were allowed to die of "old age" or were killed when obviously declining in health; the nonbreeders were killed more or less randomly at an average age of about 1 year, although about 12 per cent died. Another difference between the breeders and nonbreeders was that only two to four breeders were kept together, while the nonbreeders were kept in groups of five or occasionally more. In either case, as mice died no replacements were made.

In the genetic tests, which will be more carefully explained below, methods were similar to the above, except that no supplementary foods were given. The majority of these animals was killed at the age of about 540 days.

Necropsy was performed on all the mice except a few which had decomposed and were excluded from the statistics. Stomach tumors were recorded with an estimate of size, and many specimens were preserved for histological examination. Classification of mice as with or without a gastric tumor was based entirely on gross observations, the morphological variations not being considered.

OBSERVATIONS

In the breeder group (192 males, 292 females) the median age at death is the same in both sexes—about 535 days, or nearly a year and a half. In nonbreeding males (413 mice) the median is 540 days,
while in nonbreeding females (591 mice) the median is 392 days. The sex difference appears to have been due to a tendency to kill males first, although there was no intent to produce such a result, and it has no significant effect on the conclusions.

The incidence of stomach tumors at successive ages among mice of the four groups is presented in Chart 1. A strikingly lower frequency is shown throughout by the breeder females, while the other three groupings overlap or differ little among themselves up to the age of 400 days. After 400 days a moderate sex difference is also apparent in the nonbreeders.

Stomach tumors were rarely found in the mice that died when less than 6 months of age, but the frequency rapidly rises to a plateau by the age of about 9 months, except in the breeding females, where a rise is not obvious until the age of about 1 year. In the oldest age groups there is a slight reduction in gastric tumor frequency among breeders of both sexes.

An attempt has been made to analyze the correlation of tumor size and age at death. The data for nonbreeders afford the most satisfactory basis. Tumor size was determined for the solid part, cysts being ignored for this purpose. The estimates are admittedly rough but on the whole fairly reliable. The correlation is positive but low; many old mice had small tumors (about 2 or 3 mm. thick), while a few mice already had large tumors (a centimeter or more in diameter) at the age of 9 months (Chart 2). The median age for mice having the smallest tumors is about 340 days, while for mice having the largest tumors the median age is 400 days. Similarly, the median sizes of gastric tumors for mice less than a year old is in the small or medium-small range, while for mice over 1 year old the median tumor size is consistently greater.

There is some indication of a relation between the tumor and general health. In the nonbreeders, 74 mice died at an age of 200 days or older. Of these, only about 25 per cent had gastric tumors, a much lower incidence than for the group as a whole. In castrated Br-S mice of both sexes (6), increased incidence of gastric tumors has been reported and also a greater incidence of gastritis. However, the health of these mice has seemed good, and in mice of the Br-S strain it has been commonly noted that large gastric tumors tend to occur in large fat mice.

Genetic linkage tests.—An outcross has been undertaken to further our knowledge of the genetic basis of gastric lesions in mice of the Br-S strain. In a previous study, Strong (7) found that a majority of F1 mice from an outcross of Br-S mice (treated with methylcholanthrene) to C57 blacks had gastric tumors, and in the F1 generation close association of gastric tumor occurrence with the gene for brown coat color was observed. The conclusion drawn was that a single dominant gene was responsible for gastric tumors and that it was on the same chromosome with brown.

The mice in the study by Strong (7) were all injected with methylcholanthrene, while in the present test none were subjected to this carcinogen. The genetic formula for the Br-S mice is $\frac{a}{a}$ or $\frac{a}{a+b}$, being the nonagouti pattern; b, brown; and G, the presumed dominant gene for gastric tumors. The division line separates genes of maternal and of paternal derivation. A multiple linkage test was arranged as follows. A stock of mice related slightly to Br-S was developed with the formula $\frac{a}{a+b+}$, $\frac{a}{a+b+}$ or $\frac{a}{a+b+}$. This stock derived W (mutant dominant spotting) from strain N, where it had appeared as a mutation following methylcholanthrene injections. The heterozygote shows whitening effects in combination with other spotting factors but is slightly different from ordinary W when on a wild-type background, since the former produces hardly any white areas but, rather, scattered white hairs and a lightening of the yellow band in the hair. The homozygote, like that of ordinary W, is very anemic, white, and lives only a few days after birth. This mutant was combined with p (pink eye), another methylcholanthrene-induced mutation (recessive) in the CHI strain. Wavy, another dominant mutation, occurred spontaneously in strain JK. Whether it is the same as previously known wavy-1 or wavy-2 of other laboratories is not known. The gene se (short ear) was characteristic of the JK strain. The resulting stock was predominantly of CHI origin, since back-crosses to that strain were made. No stomach tumors have been observed in mice of CHI strain.

Reciprocal crosses of Br-S with this stock were made, and multiple-heterozygote F1 mice were selected—chiefly males. These were immediately mated with JK females, a multiple recessive type. The formulation of the cross is as follows:

\[
Wp \quad \text{Wavy} \quad se + + + \times +p+se \quad a \quad b + \quad +p+se \quad a \quad b +
\]

As it turned out, most of the selected multiple-heterozygote F1 mice did not get the se gene, so that linkage data with this gene are limited.

In addition to the above test crosses, a few mice were obtained in another test. A stock with wavy hair and d (dilution) was made up from a cross of wavy JK x C121, with selection in F2. A male wavy dilute was next mated with Br-S females, and wavy offspring were selected (three females). These
CHART 1.—Incidence of gastric tumors in mice of Br-S untreated strain according to sex, age, and breeding history. Groupings for age are by 100-day intervals or 50 days (non-breeders). Standard errors of the percentage incidence are noted for several points.

CHART 2.—A correlation table for age at necropsy and size of gastric tumor in nonbreeders of strain Br-S (untreated). The sexes have been combined. I, II, III, IV, and V are categories of tumor size from small to large. The numbers in the squares are frequencies. The regression lines are formed by connecting the medians of the rows and columns.
were then mated with a male of strain I. The formulation of this cross is as follows:

\[
Wavy \, d^{++}++ \times + d \, a \, b \, + \, p \, s
\]

As far as possible, the data from this test have been added in with the previous data. The genes \(se\) and \(d\) are closely linked so that their possible linkage relations to the gastric tumor factor should be almost identical and may be added together.

Except for the parental mice, which were breeders, practically all the animals were segregated as to sex and were kept in groups of six or fewer per box. A few escaped or died of other causes, but, out of 285 animals weaned, 251 were usable for necropsy at the required age. No mouse was included that died at 1 year or less of age, and in most cases they were killed at the age of 18 months. It is felt, therefore, that plenty of time had been afforded for tumors to develop.

The only difficulty in classification was with \(W\). Accuracy in certain combinations is very high, while in combinations with \(p\) accuracy is poor, a number of these mice being unclassifiable for \(W\). These dubious cases were arbitrarily thrown into one or the other category. One may assume about 10 per cent error.

The observed categories are given in Table 1. No definite evidence of linkage is seen in any test.

### Table 1

**Linkage Test Data Involving \(G\) (Gastric Tumor Factor)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Non-cross-over (G)</th>
<th>Non-cross-over not (G)</th>
<th>Cross-over (G)</th>
<th>Cross-over not (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G) and (d)</td>
<td>27</td>
<td>84</td>
<td>38</td>
<td>102</td>
</tr>
<tr>
<td>(G) and (a)</td>
<td>25</td>
<td>88</td>
<td>36</td>
<td>97</td>
</tr>
<tr>
<td>(G) and (W)</td>
<td>40</td>
<td>89</td>
<td>35</td>
<td>97</td>
</tr>
<tr>
<td>(G) and (Wavy)</td>
<td>37</td>
<td>86</td>
<td>24</td>
<td>85</td>
</tr>
<tr>
<td>(G) and (W) or (d)</td>
<td>9</td>
<td>13</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Totals</td>
<td>180</td>
<td>457</td>
<td>158</td>
<td>478</td>
</tr>
<tr>
<td>Per cent</td>
<td>14.1</td>
<td>35.9</td>
<td>12.4</td>
<td>37.6</td>
</tr>
</tbody>
</table>

Obviously, the proportion of animals with gastric tumors is considerably below the theoretical expectation of 50 per cent (segregation of a single dominant gene), so that about a quarter of the animals was presumably misclassified as normal. Such an error is not serious for the purposes of the linkage tests, however, as the errors in the cross-over group and the noncross-over group are simply exchanged and do not greatly affect the final percentage of crossing over.

The "penetrance" of gastric tumor (= percentage of animals that show the trait) is roughly about 70 per cent in the Br-S strain after 200 days of age (if we assume that all the mice were potentially tumor-producers). In the \(F_1\) generation from \(\varphi\) Br-S \(\times \varphi Wavy\) \(pp\) or wavy \(dd\) there were eight mice, of which three had gastric tumors (one of three females and two of five males). In the reciprocal cross of sixteen mice there were four cases of gastric tumor (two of five females and two of eleven males). On genetic grounds we should expect nearly as high an incidence here as in Br-S mice, but it is little over a third as high. In the linkage-test mice the total incidence is 26.5 per cent, while we might expect a maximum of 50 per cent. A breakdown of this generation, according to whether the parental \(F_1\) mouse had gastric tumor or not, is presented in Table 2; in the former case,

### Table 2

**Frequencies of Gastric Tumors in the Linkage Test According to the \(F_1\) Individuals from Which the Descendants Came**

<table>
<thead>
<tr>
<th>(F_1)</th>
<th>(\varphi) Normal</th>
<th>(\varphi) Tumor</th>
<th>(\varphi) Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\varphi A^*)</td>
<td>8</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>(\varphi B^*)</td>
<td>27</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>(\varphi C)</td>
<td>10</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>(\varphi D)</td>
<td>14</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>(\varphi E)</td>
<td>7</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>(\varphi F)</td>
<td>9</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>(\varphi G^*)</td>
<td>10</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>(\varphi H)</td>
<td>1</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>(\varphi)</td>
<td>3</td>
<td>3</td>
<td>30</td>
</tr>
</tbody>
</table>

\* With gastric tumor at necropsy.

DISCUSSION

In the study of Smith and Strong (6) it was observed that only 6 of 50 intact males autopsied between the ages of 200 and 600 days had gastric tumors, and of 42 intact females (nonbreeders) 29 per cent developed tumors, and in the latter, 21 per cent. The difference is not great and amounts to less than twice the standard error.

Tumor sizes in the linkage-test mice were, in general, small to medium; the incidence of large examples was much lower than in the Br-S mice.

The relation of gastric tumor to sex in the linkage-test mice is of interest. Of the 51 mice, 19 were males and 32 females, but of the 65 with the tumor, 41 were males (63.1 per cent). The sex difference here is significant. It will be noted that a similar deviation was true of the Br-S nonbreeders after the age of 400 days (Chart 1).

The relation of body weight to gastric tumor incidence was taken up more carefully in the linkage-test population. Statistical data are shown in Chart 3. In spite of similar incidence of tumors, the \(F_1\) mice were markedly larger than the later generation. No weight difference of any significance could be found between mice with or without a gastric tumor.
seven had tumors in the same age range. These incidences are strikingly lower than those in the present study, and they require comment. The stocks used were slightly different, to be sure, but both were Br-S, and incidences in methylcholanthrene-treated Br-S mice have generally been high (excluding mice which died early of a local tumor). The chief difference between the conditions in the experiment of Smith and Strong (6) and those of the present study appears to be diet. In the former case Purina Laboratory Chow was used, while in our present case the staple food was Nurishmix (Pratt Food Company, Philadelphia), with some grain, bread, and milk supplement. A

includes early mortality and lowered body weight. Large, fat mice do not necessarily show greater incidence of the tumor, but its size tends to be greater.

The most striking positive conclusion in this study is that breeding results in a greatly lowered incidence of gastric tumors in females of the Br-S strain. Presumably some factor or factors associated with gestation and/or lactation has inhibited the growth of the tumor; the hormones progesterone and prolactin are peculiar to breeding females. It is interesting to note, however, that breeding males show a generally lower incidence, after the age of 400 days, than that of the non-

relation of tumor incidence or development to gastric activity is indicated in the experiments of Bagshaw (2) in which vagotomy of young Br-S mice resulted in decreased incidence of the tumors. Andervont (1) noted a greater development of gastric lesions in mice of the I strain receiving a diet containing much roughage.

The association of age and tumor size suggests that these tumors may develop rather rapidly for a while, probably at about 8–10 months of age (except in female breeders), and then become quiescent. This interpretation differs somewhat from the suggestion by Smith and Strong (6) that the "lesion grows slowly."

Poor health is more likely to be associated with low incidence of gastric tumors and smaller size of those occurring. Poor health (as used here) in-breeding males. The difference seems significant, although the number of nonbreeder males at those ages is rather small; the same tendency is found in the linkage-test data but not in the data of Smith and Strong (6). The sex differences offer an analogy to the sex difference in human gastric ulcers (5), as well as that of gastric carcinoma.

Differences in health seem unlikely as an explanation of the low tumor incidence in female breeders. The gastric tumor incidence in these females does not rise until the age of about a year. This age is a rather common division point between normal reproduction and cessation or a marked decline indicative of senility. The reciprocal relation between reproduction and gastric tumor incidence does not continue, however; one might expect the incidence in completely sterile

CHART 3.—Frequency distributions of body weights for mice in the linkage test, with gastric tumor (0) or normal (N).

The horizontal lines indicate range, the arrows indicate medians, and the cross bars indicate quartiles.
senile females to rise toward 100 per cent, but instead it is less than 50 per cent, even in the oldest group. Another point to consider is the tendency for nonbreeder (virgin) females to remain at a slightly lower weight level than breeder females.

The castrated mice in the experiment of Smith and Strong (6) developed gastric tumors much more often than the intact controls: 36 out of 71 males and 28 out of 68 females (all over 200 days old). It is possible that an incidence of 100 per cent may be achieved with castrated mice on Nurishmix diet.

The genetic data in the present study indicate that the gene (or genes) involved in susceptibility to this type of gastric tumor is dominant, but with penetrance of varying value—usually less than 70 per cent. No linkage was revealed in these tests, in contrast to results obtained by Strong (7) in a test with outcrosses to strain C57, where close linkage with b was found. Whether or not the methylcholanthrene treatment in that test was a differentiating factor is unknown and deserves further study.

The sex differences here observed give no support to an interpretation of sex-linked inheritance of gastric tumor. A physiological explanation seems much more likely.

The problems of maternal influence, as in litter seriation effects, on gastric tumor incidence remain to be explored. Litter seriation has been ignored in the present study, since most of the Br-S mice were born in very early litters, and those in the linkage test were not followed.

SUMMARY

The incidence of adenomatous neoplasia of the stomach in mice of a control sub-line of the inbred Br-S strain is reported, with reference to age, sex, and reproductive activity. The tumors rarely appear before the age of 6 months, but incidence in nonbreeders and in male breeders reaches a maximum at about a year. Breeder females show a marked lag and a much lower incidence at all ages. Size of tumor was somewhat correlated with age, although tumors of all sizes occurred at any age after about 10 months.

An outcross of this strain was made to obtain genetic linkage tests involving the genes a, b, d, and se, p, W, and a wavy-hair mutant. The incidence of gastric tumors was uniform in all classes, so that no linkage was indicated. This result contrasts with an earlier report of close linkage of b with the gastric tumor factor in tests with mice of the methylcholanthrene-treated line. Dominant inheritance with incomplete penetrance is indicated. In the present linkage-test population no relation of tumor incidence to body weight could be detected.

REFERENCES

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