Anatomy. — The anatomy of the mammary gland in mice with regard to the degree of its disposition for cancer 1). By P. J. VAN GULIK and R. KORTEWEG. (Communicated by Prof. M. W. WOERDEMAN.)

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I. Introduction.

In 1933 LITTLE c.s. in America and KORTEWEG in the Netherlands proved that the disposition for cancer of the mammary gland in female mice is largely determined by an influence (a so-called extrachromosomal factor) on the young ones, emanating from the mother, but not from the father. (An indication in this direction which, owing to the, at that time still imperfect technique, could not be elaborated, had already been given by LATHROP and LOEB as early as 1918).

As in our breeding-colony the father also invariably stays with the young ones, only the following influences, exclusively working from the mother’s side, are taken into consideration:

1. the composition of the cytoplasma of the ovum;
2. the influence emanating from the mother-animal on the young ones while staying in utero;
3. the composition of the milk sucked by the young ones.

That however also a purely genetic (chromosomal) factor makes its influence felt in determining the degree of this disposition was emphatically argued by KORTEWEG in 1936 (1).

In 1936 BITTNER demonstrated that the extrachromosomal factor might be identical with the influence emanating from the milk on the young ones (2). As soon as possible after their birth BITTNER had separated the young ones of a cancer-strain from their mothers, and had put them with suckling mice of a non-cancer-strain. Later on most of these foster-nursed mice did not get cancer in spite of all expectations. Similar experiments on a larger scale were made by BITTNER, ANDERVONT and KORTEWEG. This paper relates to these experiments and to an extensive anatomical investigation of the mice involved.

II. Materials.

Our experiments were made on specimens of three different inbred strains of mice and on hybrids of these strains:

1) From the Laboratory of the Antoni van Leeuwenhoekhuis (Netherlands Institute for Cancer Research).

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1. the dilute brown strain Murray—Little (called K-strain by us);
2. the black strain C 57 Little (by us called G-strain);
3. the O 20 Leeuwenhoekhuis-strain (bred by us for 20 generations by means of brother-to-sister mating, and called by us O-strain).

We exclusively used virginal female mice (in male mice cancer of the mammary gland practically does not occur). By “cancer” we mean in this paper “cancer of the mammary gland”.

In the K-strain 85 %, in the O-strain 1 % of the virginal females get cancer of the mammary gland. In the G-strain this type of cancer was only once found in several hundreds of animals.

III. Influence of the milk and of the genetic composition on the frequency of cancer of the mammary gland.

A. Influence of the milk.

Table I shows our results with mice nursed by their own mother or foster-nursed. Of K-females, nursed by their own mother, 85 % get cancer

<table>
<thead>
<tr>
<th>Type of mice</th>
<th>Normal or controls</th>
<th>Foster-nursed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of mice</td>
<td>Number</td>
</tr>
<tr>
<td></td>
<td></td>
<td>developing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>breast cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>developing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>breast cancer</td>
</tr>
<tr>
<td>K</td>
<td>168</td>
<td>142</td>
</tr>
<tr>
<td>G</td>
<td>194</td>
<td>0</td>
</tr>
<tr>
<td>F₁ K ♀ × G ♂</td>
<td>335</td>
<td>232</td>
</tr>
<tr>
<td>F₁ G ♀ × K ♂</td>
<td>722</td>
<td>11</td>
</tr>
<tr>
<td>O₂₀</td>
<td>272</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>101</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>1</td>
</tr>
</tbody>
</table>

Later on: if K-females are brought up by a foster-mother of the non-cancer strain G, this percentage falls to 9 %. Whilst the mother-nursed G-females practically never get cancer later on, 13 % of them get cancer when brought up on the milk of a K-strain foster-mother.

Of the F₁-hybrids between these strains, whose mothers belong to the K-strain, 69 % respectively 4 % get cancer, according to their having been suckled by their own mother or by a G-foster-mother. Of the reciprocal F₁ hybrids bred by mothers belonging to the G-strain, 1½ % get cancer, and 46 % of those fostered by a K-foster-mother.

These results obtained by us, mainly corroborate Bittners (3) and Andervonts (4), and they convincingly prove the very great influence
which the milk, on which the mice are bred, exercises on the degree of their disposition for cancer of the mammary gland.

B. Influence of the genetic composition.

When comparing the normal K-females with the $F_1$-females $K\varnothing \times G\delta$, which two types of mice had a K-mother, we see that the cancer percentage is significantly higher when also the father is a K-animal (85% ± 2.8) than when the latter belongs to the G-non-cancer strain (69% ± 2.5). Here the influence of the father on the disposition for cancer is proved; this influence cannot be but a purely genetic one. The percentages we found in our foster-nursed females of the same groups: 9% ± 2.7 and 4% ± 2.9 are not significantly different, although pointing in the same direction.

On comparing the fostered G-females with the fostered $F_1$-females whose mothers belonged to the G-strain, we found 13% ± 4.1 resp. 46% ± 5.2, a statistically significant difference, from which also the influence of the paternal, consequently purely genetic factor, appears.

In backcrosses from one strain to the other we found the following facts:

Starting from K-females and regularly crossing them and their female descendants in the succeeding generations with G-males, it appeared that of 101 consequently 11 times backcrossed females (by us called $\pi_{10}$) only one got cancer of the mammary gland. After having been backcrossed so repeatedly these mice have practically got the genetic composition of G-mice. The influence of the extrachromosomal factor on the disposition for cancer has been entirely lost in them.

In backcrossings of G-females to the K-strain, only one out of the 95, eleven times backcrossed, females (called by us $-\pi_{16}$) appeared to have got cancer of the mammary gland. Although these mice have practically obtained the genetic composition of the cancer-strain, yet the disposition for cancer of the mammary gland is very low in them.

The genetic composition for the disposition for cancer of the mammary gland is apparently of little importance if not one or more extrachromosomal factors are active too. In case of presence of these extrachromosomal factors there is no doubt about the significance of the genetic composition.

In the K-strain in which the extrachromosomal factor and the chromosomal factor exercise their influence together again and again, the disposition for cancer of the mammary gland, as a result of these factors, has remained very high for more than 80 generations.

In backcrossing G-females to the K-strain, the disposition remains low, even after 11 backcrossings (our $-\pi_{10}$ animals), though genetically these animals practically belong to the K-strain. From this we may conclude that the extrachromosomal factor is not in the last instance a product of the genetic composition, but that the latter is independent of it.

In backcrossing K-females to the G-strain the disposition has, after 11 backcrossings (our $\pi_{10}$ animals) become very low, even lower than in
our G-mice fostered by a K-female. From this we may conclude that the extrachromosomal factor becomes inactive after a number of generations when the chromosomal factor is not present at the same time.

IV. Architecture of the mammary gland in our mice.

After the method of Vintemberger (5), slightly modified by the authors, the second mammary gland on one side was dissected, stained in hemalum and mounted in balsam of Canada. (The corresponding second mammary gland on the other side and the inguinal mammary glands were embedded for histological examination. The authors will report on this later on). Of every dissected mammary gland an enlarged drawing was made with a projection-apparatus.

A. Normal mice.

Fig. 1 presents the mammary glands of a G-strain and of a K-strain mouse of 8 months old. When killed both mice were in the same stage of the di-estrus. The architecture of the mammary gland of the G-strain mouse recalls the picture of a tree in winter; the mammary gland of the K-strain mouse resembles a budding tree in spring. The primary duct in the G-strain mouse (fig. 2) merely shows widening and buds in the second half, starting from the teat, whereas the beginnings of the secondary ducts show buds nor widening hardly anywhere. In contrast with this we find in the K-strain mouse (fig. 3) similar widening and buds along the entire length of the primary duct and also at the beginning of the secondary ducts.
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Fig. 2. Primary duct and beginning of secondary ducts of second mammary gland of a normal G-strain mouse.

Fig. 3. Primary duct and beginning of secondary ducts of second mammary gland of a normal K-strain mouse. The primary duct has buds all over its total length. Note the striking difference with fig. 2.

Fig. 5. Part of second mammary gland of a normal K-strain mouse with nodules of hyperplastic breast tissue (GARDNER).

Fig. 8. Part of second mammary gland of a G-female foster-nursed by K-female. In this photo two different types of milk ducts are to be seen, viz. K-milk ducts inoculated on G-milk ducts.

Fig. 10. Part of second mammary gland of F1 G♀ × K♂ hybrid foster-nursed by K-female. Note the appearance of nodules in inoculated K-milk ducts on G-gland-tree.

Fig. 4 presents a drawing of the second mammary gland of a nine months old O-strain mouse in the di-estrus stage. The architecture of its gland-tree is between that of the G-strain and the K-strain. The primary duct is longer and shows many small buds along its entire length; the beginning of the secondary ducts is nearly devoid of similar buds.

In a great number of specimens of these three strains the above anatomical characteristics appeared to be constantly present. The characteristic strain-differences of the primary duct were already typically present in new-born mice.

Our study being in progress, Gardner and his co-workers (6) published an interesting paper. They examined the second mammary gland of non-virginal mice of different strains. The authors mentioned noticed in the mammary gland of cancer-strain mice (the same strain which we examined), when the animals were getting older, nodules of hyperplastic mammary tissue. The frequency of these nodules, which in a sense may be considered a preliminary stage of cancer, increases as the mice have advanced further into the “cancer-age”. On the basis of our material we can, as far as virginal mice are concerned, fully confirm Gardner's discovery. (Fig. 5.)

Gardner c.s. did not notice the differences in architecture of the mammary gland in the various strains; yet these differences are distinctly visible in the photos reproduced by them.

In the normal F₁-hybrids of which the mother belonged to the K-strain, the gland-tree principally represents the K-type, but it also slightly resembles the G-type (Fig. 6 b). In the normal F₁-hybrids of which the mother belonged to the G-strain the gland-tree shows the G-type, slightly modified in the direction of the K-type (Fig. 6 a).

In these F₁-hybrids the primary duct represents the type characteristic of the strain to which the mother belonged ¹).

B. Foster-nursed mice.

In the K-females fostered by G-females (Fig. 7 b), the primary duct shows the normal K-type; the gland-tree however possesses the G-aspect.

¹) In both species of backcrossed mice (\(\tau_{10}\) and \(\tau_{10}\)) the gland-tree represents the G-type, in \(\tau_{10}\) mice, started from a K-female, slightly modified in the direction of the K-type. The study of these mice is still in progress.
In the G-females fostered by K-females (Fig. 7a) the primary duct presents the normal G-type, but in the gland-tree a very remarkable change is to be observed. On the whole the architecture of the gland-tree is of the G-type, but at the same time some ducts spring from the ducts, which, in architecture are identical with that of the K-type. It looks as if here ducts of the K-type had been inoculated on a gland-tree of the G-type (Fig. 8). In these same inoculated milkducts we found the nodules described by GARDNER.
In the fostered F₁-hybrids the primary duct presents the type belonging to the mother (Fig. 9). In both types of hybrids the gland-tree is mainly of the G-type, but in the G-females fostered by K-females we again find a similar "inoculating" of milkducts of the K-type on a gland-tree of the G-type (Fig. 10).

In table II we have collected the various data.

The first column gives the types of mice, the second column the manner

<table>
<thead>
<tr>
<th>Type of mice</th>
<th>Nursed by:</th>
<th>Genetic formula</th>
<th>Extrachromosomal factors:</th>
<th>Architecture of mammm. gland</th>
<th>Tumor incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>Own mother</td>
<td>K K</td>
<td>K</td>
<td>K K</td>
<td>85 %</td>
</tr>
<tr>
<td>G</td>
<td>.. ..</td>
<td>G G</td>
<td>K</td>
<td>G G</td>
<td>0 %</td>
</tr>
<tr>
<td>F₁K♀ × G♂</td>
<td>.. ..</td>
<td>K G</td>
<td>K</td>
<td>K K</td>
<td>69 %</td>
</tr>
<tr>
<td>F₁G♀ × K♂</td>
<td>.. ..</td>
<td>G K</td>
<td>G</td>
<td>G G</td>
<td>1.5 %</td>
</tr>
<tr>
<td>K</td>
<td>G foster-mother</td>
<td>K K</td>
<td>K K</td>
<td>K</td>
<td>K</td>
</tr>
<tr>
<td>G</td>
<td>K-foster-mother</td>
<td>G G</td>
<td>G G</td>
<td>K</td>
<td>G</td>
</tr>
<tr>
<td>F₁K♀ × G♂</td>
<td>G-foster-mother</td>
<td>K G</td>
<td>K K</td>
<td>G</td>
<td>K</td>
</tr>
<tr>
<td>F₁G♀ × K♂</td>
<td>K-foster-mother</td>
<td>G K</td>
<td>G G</td>
<td>K</td>
<td>G</td>
</tr>
</tbody>
</table>
of nursing, the third the genetic formula, in other words the chromosomal factors which may be of importance for the degree of the disposition. The extrachromosomal factors: plasma of the ovum, intra-uterine influence and kind of the milk are given in the 4th, 5th and 6th columns, K resp. G meaning that the factor relating to it, originates in a K- resp. G-mouse.

In the 7th and 8th column we give the architecture of the mammary gland: in the 7th column the architecture of the primary duct with the beginning of the first ramification and in the 8th column that of the gland-tree. In the latter case the first letter indicates the principal type; G → K meaning G-type modified slightly in the direction of the K-type; G + K means G-type, in which as a foreign element, sharply distinguished from it, some milk ducts of the K-type are found. In the last column the frequency of cancer of the mammary gland is given for each of the various types of mice.

From this table it appears that the primary duct presents the pure type of the maternal animal; the reciprocal F₁-hybrids possess a different type, although their genetic formulae are identical. From the fact that the nature of the milk is immaterial for the architecture of the primary duct (foster-nursing makes no difference) it follows that the influence of plasma and uterus, or one of them must be responsible for this architecture. (As these two factors, according to the method applied by us, are invariably both derived from the same animal, the significance of each of these factors cannot be settled by us).

The architecture of the gland-tree in pure K-mice differs from that of F₁-hybrids K♀ × G♂. In these two the genetic composition differs, whilst the nature of the extrachromosomal factors is the same. A similar difference in architecture exists between pure G-mice and the F₁-hybrids G♀ × K♂. From this it follows that the architecture of the gland-tree is determined, at least partly, by purely genetic influences.

From our table it appears however that the influence of the extrachromosomal factors on the architecture of the gland-tree is of much greater importance: in K-mice, fostered by G-females, the K-type to be expected, under the influence of the G-milk, has been altered into the G-type. Only in case all three extrachromosomal factors are derived from a K-mouse, does the gland-tree show a genuine K-type: if only the milk or only plasma + uterine influence of the K-animal have been at work, the gland-tree shows a — possibly more or less modified — G-type (compare normal K with fostered K, normal F₁ K♀ × G♂ with fostered F₁ K♀ × G♂ and normal F₁ K♀ × G♂ with fostered F₁ G♀ × K♂). Both the kind of milk and the nature of plasma- and uterusfactor consequently influence the architecture of the gland-tree.

In the two types foster-nursed by a K-mouse (G by K and F₁ G♀ × K♂ by K) inoculation of K-ducks on the G-gland-tree has taken place, as a distinctly foreign element.
V. The anatomical architecture of the mammary gland as an explanation for the degree of its disposition for cancer.

From table II it appears that the only gland-tree showing the genuine K-type (normal K-females) possesses the highest disposition for cancer by far. Next comes, concerning the degree of this disposition, the only other gland-tree of the K-type already slightly modified however in the direction of the G-type (normal F₁ K♀ × G♂ hybrids). Hereupon follow both gland-trees of the G-type which show inoculated genuine K-ducts (G-females foster-nursed by K-females; F₁ G♀ × K♂ hybrids foster-nursed by K-females). The disposition of the G-gland-trees, which have been slightly modified in the direction of the K-type but still possess no genuine K-type ducts is still lower (F₁ K♀ × G♂ hybrids foster-nursed by G-females; normal F₁ G♀ × K♂ hybrids).

The disposition of the gland-tree of the genuine G-type is extremely low. (In K-females fostered by G-females, with 9% cancer, we found a gland-tree of the G-type. This apparent contradiction may be explained by the fact that of these mice only a very small number have been examined).

From our material it most convincingly appears that the degree of the disposition for cancer of the mammary gland is closely connected with the anatomical architecture of this organ.

VI. Summary.

The factors determining the degree of disposition for cancer of the mammary gland in the mouse are discussed on the basis of the result of extensive breeding experiments. In a great number of mice the anatomical architecture of the mammary gland was examined. The relation existing between this architecture and the disposition for cancer of the mammary gland is discussed.

VII. Conclusions.

1. There exist typical differences in architecture of the mammary gland between the mice-strains examined by the authors.
2. This architecture is influenced both by genetic and extrachromosomal factors.
3. The architecture of the primary duct is determined by the plasma- and (or) uterus-factor.
4. The architecture of the gland-tree is determined both by the genetic and by the extrachromosomal factors: milk, plasma- and uterus-factor.
5. There is a strong positive correlation between the architecture of the mammary gland in different types of mice with regard to the degree of its disposition for cancer.
LITERATURE.

1. KORTEWEG, R., Genetica 18, 350 (1936).
5. VIENTEMBERGER, P., Arch. de biol. 35, 125 (1925).