Fluorescent Porphyrins in Harderian Glands and Susceptibility to Spontaneous Mammary Carcinoma in Mice*

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During the quantitative determination of the xanthine oxidase (dehydrogenase) activity in livers of mice of mammary cancer-susceptible and cancer-resistant strains (11), the unused parts of the mice were dissected and examined under near-ultraviolet light. One motive for doing this was related to the fact that one of us had been collaborating with another group, attempting to trace the glandular source of the red fluorescent exudate which had accumulated on the nose and whiskers of pantothenic acid-deficient rats (22). During this work, the very brilliant red fluorescence of the Harderian glands of rats was observed. Mainly out of curiosity, Harderian and lacrimal glands and contents of orbital cavities of all available animals were examined in near-ultraviolet light. No red fluorescence was observed in the contents of the orbital cavities of monkeys, dogs, cats, squirrels, rabbits, guinea pigs, or an opossum.

Mice of the JK cancer-resistant strain and the C3H cancer-susceptible strain were available from the xanthine oxidase (dehydrogenase) determinations. When the Harderian glands from the mice of these two strains were dissected in near-ultraviolet light or so-called “black light” (General Electric Type B-H4 lamp) a remarkable observation was made. The Harderian glands of the C3H mammary cancer-susceptible mouse exhibited a red fluorescence, but the glands of the JK mammary cancer-resistant mouse fluoresced pale white. These procedures and observations were repeated 3 more times with exactly the same results before it was decided to investigate the phenomenon more carefully.

In rats, the red fluorescence of the Harderian glands had been shown to be due to the presence of a protoporphyrin (7, 22). In the case of the mouse Harderian gland, the red fluorescent material is in all probability also a porphyrin. It was soluble in acetic acid and when this was neutralized, it could be extracted with ether. It was removed from the ether with 8 per cent hydrochloric acid but not with 0.5 per cent. It was also soluble in chloroform. The close phylogenetic relationship between rats and mice, the shade of red fluorescence of the material, and the solubility characteristics made it reasonable to assume that the red fluorescence was due to a porphyrin. It is probably mostly protoporphyrin, but until this has been analyzed more thoroughly it is unsafe to be more specific.

A direct relationship between the red fluorescence of the Harderian gland (a lacrimal gland) and cancer susceptibility was regarded as highly improbable. The striking difference in the red porphyrin fluorescence of this gland in mice of cancer-susceptible and cancer-resistant strains was taken to indicate a difference in porphyrin metabolism in mice of the two strains. That such a difference in porphyrin metabolism might be directly or indirectly related to cancer susceptibility is not regarded as improbable for the reasons that will be apparent from a brief consideration of porphyrins.

Porphyrins occur in practically all forms of life (both plants and animals) that exhibit normal and atypical growth. This is because chlorophyll and hemoglobin and similar large molecules contain porphyrins as active constituents. Some of the most important respiratory enzymes such as cytochrome c, catalase, the Pasteur enzyme, and peroxidase also contain porphyrins as active parts of the molecule (19, 27, 28, 40, 41). The fundamental nature of the relationship of all of these substances to cellular physiology, especially to oxidations and reductions, cannot be questioned.

From these initial observations and considerations, it appeared that there might be some direct or indirect relationship between susceptibility to spontaneous mammary carcinoma and porphyrin metabolism, or factors regulating this. The first step which has been taken to investigate this possibility was to see if this observation could be extended to mice of other strains in which the degree of susceptibility to spontaneous mammary cancer was known to vary quantitatively from that found in C3H female mice. As mice from strain after strain were examined, the observed intensity of the red fluorescence of the Harderian gland paralleled the previously established degree of susceptibility to cancer. The large number of animals examined, the number of strains analyzed, and the close parallelism observed, indicate that this association was probably not due to coincidence. The preliminary report described the observations on a limited number of C3H and JK mice (34). The extension of the examinations and analysis to include mice in other strains will be described here.

MATERIALS AND METHODS

The mice used in this investigation were from the colony of L. C. Strong. It was desirable for at least two reasons to make a survey of as many strains of...
mice as possible: 1. in order to ascertain whether or not there was a significant degree of variability in the fluorescence of the exposed Harderian glands of mice of the different strains, and 2. to see if this variability paralleled the variation in susceptibility to cancer. Mice of 13 inbred strains and of 3 hybrid lines were used. These are listed in Table I.

It should be noted that these 13 inbred strains can be classified into 3 groups depending upon the degree of susceptibility to spontaneous cancer of the mammary gland of the females. High susceptibility to spontaneous breast cancer is found in female mice of the C3H and A strains; intermediate degrees of susceptibility in female mice of the C, CI2I, CHI, CBA strains; low susceptibility or complete resistance in female mice of the JK, I, F, C57, N, and L strains. F mice, however, give rise to spontaneous myelogenous and lymphatic leukemia as indicated by the work of Kirschbaum and Strong (21). Of the hybrid mice used, not enough is known of their tendency to develop spontaneous tumors of breast tissue to warrant classification into one of these 3 classes. The incidence of spontaneous mammary cancer in NH and NHO mice is, up to the present time, nonexistent. In addition to this resistance to spontaneous tumors, the NHO mice have been produced by selection toward a high resistance to induced tumors by methylcholanthrene. The FC mice have, so far, never developed spontaneous tumors of any kind.

TABLE I: LIST OF STRAINS OF MICE USED IN EXPERIMENTS

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inbred generations</th>
<th>Number of mice used</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>56</td>
<td>275</td>
</tr>
<tr>
<td>A</td>
<td>74</td>
<td>48</td>
</tr>
<tr>
<td>JK</td>
<td>49</td>
<td>68</td>
</tr>
<tr>
<td>I</td>
<td>35</td>
<td>42</td>
</tr>
<tr>
<td>CI2I</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>F</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td>N</td>
<td>36</td>
<td>26</td>
</tr>
<tr>
<td>CHI</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>CBA</td>
<td>52</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>51</td>
<td>14</td>
</tr>
<tr>
<td>CBAN</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>L</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>C57</td>
<td>16</td>
<td>52</td>
</tr>
<tr>
<td>NH (unselected)</td>
<td>13</td>
<td>80</td>
</tr>
<tr>
<td>NHO (selected)</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>FC</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>BC to L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total 753

* All stocks, except C57, were originated and maintained by L. C. Strong. The C3H was obtained from Dr. H. J. Bagg of New York City and continued in this laboratory for the past 3 years. The unselected NH strain was developed without selection from an original cross between mice of the CBAN and JK strains. The selected NHO strain was established by selection toward resistance to tumors induced by methylcholanthrene. The selected FC strain was obtained by a cross between mice of the F and C57 strains. The BC to L mice were produced by a backcross to the L strain mice from an original cross between L and JK mice.

All mice were maintained on a diet of Nurishmix and water ad libitum and given fresh, washed lettuce twice a week. They were killed either by pinching the spinal cord in the cervical region between the thumb and forefinger or by means of illuminating gas. The skin over the eye was cut away by fine scissors and the eyeball extracted by pulling it out with forceps. The Harderian gland was then grasped with a pair of fine forceps and pulled away from its loose attachments in the orbital cavity. It was then placed on blotting paper where all traces of blood and connective tissue were removed. The glands were then stretched on a clean microscopic slide and pressed down slightly with forceps. All glands were then examined under near-ultraviolet light (General Electric Type B-H4 “black” lamp) within a few minutes after killing.

A method for quantitative determination of the intensity of the red fluorescence of a single gland has not as yet been devised. In this investigation, semi-quantitative estimates of the degree of red fluorescence of Harderian glands were made by visual inspection and comparison. The current was turned on several minutes before the examination and estimation in order to permit the lamp to reach its maximal intensity. In nearly all cases, however, mice from many stocks were examined at the same time, the Harderian glands of 25 to 30 mice being placed on the same microscope slide. By these procedures, the personal element and the possible fluctuations in the source of near-ultraviolet light were somewhat controlled.

In order to evaluate the different degrees of fluorescence encountered, an arbitrary division into 6 classes was set up (one more than was used in the preliminary report). These were o, +, ++, ++++, +++++, +++++. The o class was used to designate the absence of all traces of red fluorescence. These glands appeared to fluoresce white or light tan in near-ultraviolet light. Occasionally a tiny spot of pink fluorescence would show in one end of the Harderian gland. The maximum degree of fluorescence was designated as +++++++; here the red fluorescence was very intense and was comparable to the condition found in normal rat Harderian glands. Glands showing intermediate degrees of fluorescence were classified as +, ++, ++++, or +++++, depending upon the estimation of fluorescent intensity.

RESULTS

A great variation in the degree of red fluorescence of the Harderian glands in mice of the various strains was observed. Since the intensity of fluorescence was quite constant in the two glands of the same mouse, it was evident that they were both apparently in the same physiological state at the same time. The only variability encountered in the two glands of the same
mouse was seen occasionally in those mice which approached the 0 class; here one gland would give completely white fluorescence, whereas the other would show a small localized area of very light pink fluorescence. Mice of the same inbred stock and age showed constant degrees of fluorescence. At a given age, however, the females tended to show, on the average, a slightly greater degree of fluorescence than that of the males, but this finding was not always constant. The maximum degree of difference between mice of the different strains was found during early sexual maturity in the C<sub>3</sub>H (+++++) on one hand and in the old JK, N<sub>3</sub>, and C<sub>57</sub> (o) on the other. Harderian glands of mice of the other strains showed intermediate degrees of fluorescence (+, +++, +++, +++, and ++ + + +).

This difference between the strains was not absolute since variations with age occurred. Before the eyes were open at 14 days, there was no red fluorescence evident in the contents of the orbital cavities of any mouse. Shortly after the eyes were open there was a rapid increase in red fluorescence which reached its maximum development at approximately 100 days of life (in JK mice this maximum reading was ++ and in C<sub>3</sub>H mice, ++++++). After that time there was a rapid drop in the red fluorescence of mice of the JK strain which completely disappeared in middle sexual maturity, and this absence of red fluorescence then persisted during the rest of the life span. On the other hand, there was a much slower decline in the intensity of red fluorescence in mice of the C<sub>3</sub>H strain with advancing age. Only one individual of the C<sub>3</sub>H strain, at 29 months of life, had no red fluorescence. The changes in fluorescence with age are shown for the JK and C<sub>3</sub>H mice in Fig. 1.

In contrast to this constancy of fluorescence in individuals of the same age and inbred strains, there was considerable variability in fluorescence in individuals of the same age belonging to the hybrid lines FC, NH, and NHO. That is, when there was a high degree of genetic uniformity between individuals of the same inbred strain and age, uniformity of red fluorescence was seen. When there was marked genetic variability among the individuals (in hybrid generations following an outcross) a diversity of fluorescence was seen among the individuals.

Since a few backcross mice to the L stock (from a cross between JK and L) were available, these were examined under near-ultraviolet light. They all approached the reading of fluorescence determined in mice of the L strain, thus indicating that the presence of porphyrins causing red fluorescence of the Harderian glands may be an inherited dominant characteristic.

As an incidental finding, it was observed that the color of the Harderian glands in mice of the different strains was variable even in visible light due to the relative abundance of a dark pigment. For example, mice of the A strain (with pink eyes) have practically flesh-colored Harderian glands under visible light; mice of the C<sub>3</sub>H strain (with black eyes) have dark gray Harderian glands under the same light. This "melanin-like" pigmentation, however, is not necessarily correlated with eye color, since mice of the N strain with black eyes have flesh-colored Harderian glands. Whether or not this pigmentation or any other constituent of the Harderian gland has any effect on the presence or absence, abundance or proportion of porphyrins is not clear. Hybrid mice of the FC descent (all with black eyes) showed variable degrees of pigmentation under visible light. At least four grades could be detected in litter mates as follows: (a) flesh color, (b) light gray, (c) medium gray, and (d) dark gray. This variability was not seen in mice of the inbred strains, thus indicating that the presence of this pigment may have a genetic basis.

As a general rule, females show more of this gray pigmentation than do litter-mate males. Mice with the gene for brown show lighter Harderian glands than mice with the gene for black coat color. There is a decrease in the amount of gray pigmentation as well as red fluorescence with advancing age in both males and females after early sexual maturity.

One apparent exception to the rule that mice with high susceptibility to spontaneous cancer show maximal red fluorescence of the Harderian gland was seen in mice of the A strain. Instead of showing +++ + + +,
fluorescence, as might be expected, some of the mice showed the amount characteristic of those mice showing intermediate degrees of susceptibility (+ + or ++ +). The reason for this is unknown. Whether or not the absence of the dark visible pigment (as is observed in A strain mice) permits a rapid decomposition of the red fluorescent porphyrin of that gland has not been determined. The fluorescence in the dried Harderian gland of the C3H mouse persists longer than does the fluorescence in a gland from a mouse with an intermediate reading. This may be due to an originally greater amount of porphyrin rather than to the possibility that the substance is protected from deterioration by special conditions existing in that gland.

In contrast to the findings obtained many times on mice of other strains it was noted that the red fluorescence of the Harderian gland in mice of the A strain seemed to increase temporarily in intensity after the gland had been placed on a microscope slide for a short time. Therefore, this lower degree of red fluorescence in mice of the A strain may not be real, but may be due to some peculiar condition within the gland that masks or prevents the full manifestation of fluorescence. The Harderian glands of all mice lose their red fluorescence when kept on a microscope slide at room temperature. The capacity to give the characteristic fluorescence may be retained at least for several weeks by keeping the glands frozen.

**DISCUSSION**

In discussing the close parallelism which was found to exist between the degree of cancer susceptibility and the variations in porphyrin metabolism as indicated by different degrees of the intensity of red fluorescence of Harderian glands, two main possibilities must be considered. One is that this observed parallelism is due to mere coincidental association of these two characters which may be otherwise unrelated to each other. Such associations are sometimes observed in genetic studies. The second possibility is that some fundamental direct or indirect interrelationship may exist between these two characteristics.

The apparently paradoxical situation in the case of rats would seem to lend support to the first possibility. The Harderian gland of the rat exhibits an intense red fluorescence, yet rats rarely develop spontaneous mammary carcinoma. This fact could be regarded as evidence in favor of the hypothesis that cancer susceptibility and factors influencing porphyrin production and metabolism are independent and unrelated and that their close association in mice is merely due to a chance coincidence. However, if we assume, on the basis of the red fluorescence of the Harderian glands, that certain closely related factors which help regulate both porphyrin metabolism and cancer susceptibility are present in both rats and cancer-susceptible mice, it does not follow that there should be a high incidence of spontaneous mammary carcinoma in both. Some additional interrelated factors which influence susceptibility to cancer may be operating in the mouse and not in the rat. Likewise, other factors regulating the presence or potency of carcinogenetic stimuli may be present in the mouse and not in the rat. On logical grounds, therefore, the fact that the rat has a brilliant red fluorescent Harderian gland and yet low incidence of or resistance to spontaneous mammary carcinoma cannot be used as an argument to disprove the hypothesis that factors that regulate porphyrin metabolism are also concerned in the determination of cancer susceptibility.

The other possibility; namely, that a fundamental relationship exists between the factors regulating porphyrin metabolism and the factors determining susceptibility to cancer, is supported by the observed close parallelism and by some other meager evidence. The facts which indicate a possible direct relationship will be considered first and the more conservative concept of an indirect relationship will be mentioned last.

At first it was thought that excess porphyrins might have a direct effect on the susceptibility to a given carcinogenic stimulus. The high concentration of porphyrin in the Harderian glands of cancer-susceptible mice which is responsible for the red fluorescence, is not necessarily an indication of the porphyrin concentrations of other organs and tissues. If the Harderian gland does not elaborate the porphyrin but extracts it from the blood, it is conceivable that the continuous secretion of porphyrin by this gland would decrease rather than increase the concentration of porphyrins elsewhere. On the other hand, if the Harderian gland does elaborate the porphyrin, which is responsible for the more persistent and intense red fluorescence observed in the cancer-susceptible mouse, then it is highly probable that this gland is responsible for a generally higher porphyrin level in other organs and tissues.

This statement is made on the basis of the following observations in rats: If rats are deprived of water, the red fluorescent secretions of the Harderian glands accumulate on the noses of the rats (9). When the rats are then allowed to drink water, they soon clean the red fluorescent material off the nose and fur. Such rats, when killed 4 hours after the cleaning process, exhibit a strong red fluorescent band in the intestine due to the fluorescence of the unusually large amount of material cleaned off by washing and licking. The feces of rats are red fluorescent, due to presence of porphyrins (25). When the Harderian
glands are removed from rats (16), the feces lose the red fluorescence. These facts indicate that the Harderian gland in the normal rat, while it is presumably an excretory gland, secretes a porphyrin that probably finds its way back into the blood stream by way of the nasolacrimal duct, the nose, the nasopharynx, and the alimentary tract. If only a part of the porphyrin be absorbed from the alimentary tract, it would thus be possible for a porphyrin-elaborating Harderian gland to increase the concentration of porphyrins in other organs and tissues. The influence which the Harderian gland has on the concentration of porphyrins elsewhere depends on whether or not this substance is elaborated in the Harderian gland and this is not known.

In addition to uncertainty as to whether the secretion of porphyrins by the Harderian gland increases or decreases the concentration of porphyrins in other organs or tissues, there is little or no evidence to indicate that variations in porphyrin concentration would influence either cancer or susceptibility to cancer. Some fragmentary but suggestive data that may have a bearing on this question may be mentioned. The fact that porphyrins are present in bone marrow as a necessary step in the synthesis of hemoglobin may be relevant. Throughout life the bone marrow is one of the most active tissues as regards cellular proliferation. According to Borst and Königsdörffer (5) the megaloblasts and erythroblasts contain a relatively large amount of protoporphyrin. Porphyrins were demonstrated by fluorescence studies in the central necrotic inoculation center in induced rat sarcoma (24). This was thought, however, to be of bacterial origin. More recently, Thomas (37) has described the fluorescence of the tumor masses in cases of chloroma. As indicated by the name, these masses appear green in visible light but they are red fluorescent in near-ultraviolet light. Thomas identified the red fluorescent pigment in these tumor masses as protoporphyrin (37). In addition he was able to extract protoporphyrin from the red fluorescent centers of lymph nodes in two cases of myelogenous leukemia. This red fluorescent pigment was also extracted and proved to be protoporphyrin.

Estrogenic effects are also claimed for porphyrins by Rodewald (26) who reported that a series of several estrus stages may be induced in ovariectomized mice by a single series of injections of porphyrin. This work, however, lacks confirmation. The same author reported that injections of porphyrins stimulate the hypophysis to secrete larger amounts of melanophore-dispersing and gonadotropic hormones. In the serum of injected animals, a substance which inactivated the melanophore-dispersing hormone was demonstrated which was thought to be identical with the one found in the serum of patients with cancer (20). Dobriner and Rhoads (8) treated rabbits with 1,2,5,6-dibenzanthracene and observed increased porphyrin output in the urine. Except for the mention of the work on dibenzanthracene and porphyrin, recent reviews (8, 39) do not refer to any relationship between high porphyrin levels and cancer.

The photosensitizing action of the porphyrins, demonstrated by Hausmann (18), has been amply confirmed by numerous experiments. One of the most impressive is the experiment of Meyer-Betz (23) who injected himself with 200 mgm. of hematoporphyrin. He developed a severe edema of the skin in areas exposed to light. This photosensitivity lasted several months. In this connection, it would be of interest to determine whether the ultraviolet radiation technic used to induce cancer in albino mice would be as effective in mice with nonfluorescent Harderian glands (4).

The first working hypothesis that developed from the present data and these considerations was that excess porphyrins might influence cancer susceptibility by sensitizing cells to such agents as light, or chemicals formed by the action of light, estrogenic, carcinogenic, and perhaps other similar substances. In addition to the work on estrogenic effects of porphyrins which may be interpreted in terms of increased sensitivity to normally occurring chemicals, there are certain isolated facts that were observed in patients excreting abnormally large amounts of porphyrin, that support this view. In one case, hirsutism was observed in a female. In describing cases of acute porphyria, Grünwald (17) described a virgin who secreted colostrum. Harbitz (17) in discussing another case, described the swelling of the breast of a man during an attack of acute porphyria.

It is, probably, more conservative to postulate a relationship between the factors that regulate porphyrin metabolism and those that determine susceptibility to cancer. To introduce a new concept, such as "the variability in factors regulating porphyrin metabolism" into the already complex sequence of events involved in susceptibility to spontaneous carcinoma of the mammary gland is, perhaps, a hazardous procedure. Especially is this true when so little is definitely known about the factors which regulate the metabolism and effects of porphyrins within the body. That variations in the regulation of its production might affect hemoglobin and many of the important respiratory enzymes such as catalase, cytochrome c, and the Pasteur enzyme may be highly significant. But whether these enzymes are definitely involved in the origin of the cancer cell has not been proved. That there are variations from the normal in the above enzyme systems...
after the cancer cell has been established may be the result and not the cause of the cancer cell.

But quite aside from these fundamental studies on cell metabolism which may eventually provide the key for the interpretation of the origin of neoplastic growth, there are six established concepts which are recognized in one form or another as being involved in the explanation of susceptibility and resistance to spontaneous mammary cancer. These may be briefly classified as 1. the dietary, 2. the physiological use or forced breeding, 3. the milk influence, 4. the hormonal, 5. the chemical, and 6. the genetic.

In reference to these, two questions may be asked: (a) Are the contributions from these six fields sufficient to explain completely the sequence of events involved in cancer susceptibility and resistance? (b) Would variations in porphyrin metabolism aid in the interpretation of cancer susceptibility and resistance?

1. The role of diet in cancer susceptibility and resistance is still too little understood. It is known that the incidence of breast cancer as well as other types from other organs can be significantly influenced by changes in the diet, but these alterations are brought about by the use of commercial feeds or by the introduction into the diet of such complex materials as liver (6), etc. Until the mice are supported on synthetic diets, the role of diet or any constituent of the diet cannot be properly evaluated.

2. The physiological use factor of Bagg (1, 2) is an important influence on the incidence of breast cancer. Certainly, however, the effect of use of any part such as the breast must indeed be very complex and much more must be known of what happens to tissues during the process of physiological activity before the final evaluation of this contribution can be made. It is known that without a certain amount of genetic susceptibility, forced breeding does not necessarily increase the incidence of breast tumors. It is also true that in order to bring mice to a level of forced breeding sufficient to lead to the development of breast cancer, maximal conditions of diet must be used so that the dietary influence may be as effective in increasing the incidence of breast cancer as the physiological effects of forced breeding itself.

3. Bittner's milk influence (3) on breast cancer of mice is, perhaps, as clear cut as any influence since the end result or percentage incidence of breast cancer (due to milk supply alone) is remarkably affected. No one is willing, however, to maintain that this influence, even though it be a virus, is sufficient in itself to explain why a particular mouse develops breast cancer or not. Bittner (3) has definitely indicated that a hormonal and a genetic influence must be present in order to explain the incidence of breast tumors in mice raised by foster nursing.

4. A considerable amount of work has shown that the early stimulation of breast tissue by estrogens brings about breast cancer in male mice, providing the male belongs to a genetic strain in which the females show a high incidence of spontaneous breast tumors. But hormonal response does not completely parallel genetic susceptibility. For example, Gardner (13-15) has indicated that it is easier, by hormone injection, to bring about tumors in CBA male mice than it is in A strain males, whereas the susceptibility to spontaneous breast tumors in females of these strains is just the reverse. The doses necessary to bring about tumors by the injection of estrogen are still excessive over prolonged continuous stimulation, and may be far beyond the range found in the normal physiology of the control animal. However, with the use of chemically pure estrogens in pellet form, the chief difference between experimental and normal conditions lies in the fact of a continuous (not actual) supply of the estrogen in experimental mice rather than in the cyclic as occurs in normal female mice. On the other hand, Twombly (38) has shown that by a combination of foster nursing and hormone injections, male mice belonging to a genetically resistant-to-cancer strain may give rise to breast tumors; that is, this combination of two principles apparently produces the same effect as the chemical influence mentioned below.

5. Recently it was shown that the presence of methylcholanthrene in female mice of the NHO strain being used for breeding will bring about breast cancer in about 10 per cent of the animals. So far this phenomenon has been found only in mice belonging to a genetic strain characterized by a low incidence of spontaneous breast tumors. That is, the presence of the carcinogen replaces, as it were, genetic susceptibility. Thus, it is reasonably certain that the mechanism involved in the origin of a methylcholanthrene-induced breast cancer is not necessarily the same as the one involved in a mouse receiving estrogen alone.

6. That a genetic determiner is involved in susceptibility and resistance to spontaneous breast cancer is commonly accepted. Just what role this determiner plays is not yet clear. Many suggestions have been made, some on theoretical grounds, others on experimental evidence, of how a determiner from the germ plasm could alter or influence an individual in such a way that breast cancer will of necessity eventually develop. Among these suggestions based on experimental evidence are those of Strong (12, 29-36) and his collaborators who have endeavored to bridge the gap between the germ plasm and the disease, cancer, by an analysis of the physiological components
of the individual. These studies have shown 1. that there is a precocious drop of hemoglobin in a mouse known to be genetically susceptible to breast cancer as compared to one of a cancer-resistant strain (12, 35, 36); 2. that the mouse belonging to a susceptible-to-cancer strain is less tolerant to salicylaldehyde than one from a resistant strain (32, 33); 3. that a mouse of a strain showing high susceptibility to spontaneous breast cancer has less xanthine (oxidase) dehydrogenase activity in its liver (11); and 4. that the susceptible-to-cancer mouse also shows more intense red fluorescence of the Harderian gland (thus indicating a porphyrin metabolism of a certain type) than a mouse of a resistant-to-cancer strain (34). These seemingly unrelated phenomena may have something in common. With the exception of xanthine dehydrogenase activity of livers, where the available data do not permit a comparison as yet, they follow the same type of distribution in an individual at different age levels; a low initial reading which increases in amount during early sexual life, remaining uniform in middle sexual life, and then diminishing with advancing age. If this be true, then the genetic determiner in cancer susceptibility (of which the above criteria may be indices) may be merely influencing the aging process of individual tissues or the “individual as a whole.” Other physiological functions may also be determined later, which also vary according to this general trend with age.

It is likely, however, that the contributions of the six separate fields of biological research enumerated above and bearing upon the problem of the origin of the cancer cell are not in themselves sufficient to explain the changes involved in the conversion of a normal to a neoplastic state. Some concept such as a sensitizing effect of a chemical may be necessary to correlate some of the diverse concepts.

SUMMARY

The finding is reported that mice of strains with high susceptibility to breast cancer show more intense red porphyrin fluorescence of the Harderian glands than do mice belonging to strains with low susceptibility to cancer. The close parallelism between the degree of red fluorescence of the Harderian gland and the degree of cancer susceptibility in over 750 mice of 16 different strains was regarded as evidence in favor of the following hypothesis: There is a direct or indirect relationship between porphyrin metabolism and the factors that determine cancer susceptibility.

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