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4. The Bearing of Mouse Genetics on Our Understanding of Human Cancer[1]

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THE problems involved in our understanding of the cause, origin and development of cancer are basically problems in biology. The study of neoplasia concerns living cells and the factors acting through the physiology of the living

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organism and of the cell to cause it to take on a new characteristic of uncontrolled growth and to support this growth. Cell differentiation, cell nutrition, growth and biochemistry are involved, and, in that all of these are under the control of the genotype of the organism and of the cell, genetics is likewise involved. It is, therefore, through studies in genetics that much of our fundamental understanding of cancer is derived.

Because of difficulties in the collection and analysis of data from human beings on such a complex character as cancer, the ground work for the study of the genetics of cancer must be done with some other species. Important contributions to our understanding of the problem have been made and will continue to be made by investigations with Drosophila, corn, Neurospora and other lower organisms. Because of its physiology approximating that of man and because of its wide variety of neoplasms occurring with such frequency necessary for adequate study, the mouse, however, has provided the most extensive approach to the problem. Certain superficial facts revealed obviously can be applied only to the mouse, but basic facts and concepts derived from studies on the mouse can also be applied to man.

IS CANCER INHERITED?

From genetic studies in mice one is forced to conclude that probably every type of cancer in man can be subject to genic influence so that under certain conditions the genotype of the individual may be the deciding factor in determining whether or not the individual develops a certain type of cancer. Through the manipulation of genes in the development of the inbred strains, and through hybridization of the strains, geneticists have been able to prevent or promote the occurrence of more different types of cancer and to influence the occurrence of each to a greater degree than has any other investigator with any other group of agents. Strains vary in respect to incidence of mammary gland tumors from strain C3H in which 95 per cent or more of the females have mammary tumors to strain C57 BL in which less than one per cent of the females develop mammary tumors (Andervont, 1941, Heston *et al* 1950). Extremes in regard to pulmonary tumors are strain A with an incidence of 90 per cent at 18 months of age and strain C57 L in which only 3 spontaneous pulmonary tumors have been observed in the thousands of mice throughout the history of the strain (Heston, 1942b). Strains C58, and AKR are known for their high incidences of lymphocytic leukemia. (MacDowell, *et al.* 1945, Law, 1948). These have been reported as high as 90 per cent. Little (1941) has reported an incidence of non-epithelial tumors of about 20 per cent in strain C57 BL. Most of these were classified as lymphoblastomas. About 25 per cent of mice of strain C57 L develop a Hodgkins-like lesion. In contrast, in strains A and C3H neoplasms of the blood-forming organs are rarely found. Hepatomas occur in approximately 30 per cent of strain C3H males and 10 per cent of the females

(Andervont 1941). Strong (1945) has developed a strain with a high incidence of tumors of the glandular stomach, and strain I has a high incidence of a lesion of the glandular stomach (Andervont and Stewart, 1937), whereas tumors of the gastrointestinal tract rarely occur in other strains. Pybus and Miller (1938) reported a strain of mice with a high incidence of bone tumors, a type rarely found in other strains. Gardner and Pan (1948) reported a strain PM derived from the original Pybus and Miller strain with a high incidence of spontaneous uterine, cervical and vaginal tumors, although none were observed in 6 other strains. Woolley and co-workers (1945) developed a strain CE with a high incidence of carcinoma of the adrenals following early castration. With the same treatment strain DBA mice showed only hyperplasia of the adrenal cortex and strain C57 BL mice gave little if any adrenal response. Burdette and Strong (1943) reported genetic differences in the incidence of subcutaneous sarcomas following the injection of methylcholanthrene. Strain $C3H_f$ mice without the milk agent and their hybrid derivatives have a higher incidence of spontaneous subcutaneous sarcomas than have other strains. Mice of strain HR which carries the hairless gene have a relatively high incidence of spontaneous papillomas and when painted with methylcholanthrene develop a higher incidence of squamous-cell carcinomas than has been reported for any other strain (Deringer, 1951). Even in respect to very rare tumors such differences are seen. Myoepitheliomas, often of salivary gland origin, occur much more frequently in strain A than in any other strain. In hybrids between strains C3H and C57 BL a number of tumors of the Harderian gland were noted although this tumor has not been reported in any inbred strain or other type of hybrid.

From these observations of genetic differences in respect to these many varied types of tumors it is safe to assume that strains of mice differing in incidence of any type of tumor could be developed with the proper genes in the original population followed by the necessary selection.

One who has worked with these strains of mice, therefore, no longer asks if cancer is inhreited in man. Instead he asks how is cancer inherited in man and how important are the genetic factors as compared with the various non-genetic factors in determining whether or not cancer will occur.

CAN A GENERAL INHERITED SUSCEPTIBILITY TO CANCER IN MAN BE EXPECTED?

One of the first questions to be answered by data from the inbred strains is whether or not a general susceptibility to cancer is inherited. The fact that the strains are susceptible to specific types of cancer and that almost no strain is resistant to all types has indicated that the various types are inherited as independent characteristics. For the most part this would be expected when one visualizes the physiologic paths through which the genic action must become manifest. It is difficult to perceive how the development of pulmonary

tumors could be preceded by the same physiologic factors that affect mammary gland tumor development.

On the other hand it would seem possible that the development of tumors in different organs of the same physiological system might have a common genetic basis. Variations in hormonal balance might influence tumor development in a number of endocrine organs, the reproductive organs and the breast. There is some evidence of a relationship between adrenal hyperplasia and the genes influencing mammary tumors through the control of the hormonal stimulation in mice. (Smith 1948, Huseby and Bittner 1948). Data of Macklin (1952) suggest that such an association may be demonstrated in man in the relationship between breast cancer and cancer of the prostate. In the mouse specific genes have been shown to influence the development of more than one tumor, but, as will be discussed subsequently, the effect may be through separate physiologic paths and one gene may have opposite effects upon two different types of tumor.

MULTIPLE FACTOR INHERITANCE OF CANCER

Another major question to be answered is in regard to the type of inheritance observed in respect to cancer. It should be emphasized that in studies of the mouse where breeding experiments can be carried out and where F_2 and backcross segregants can be tested to differentiate between single factor ratios and simulated single factor ratios due to fluctuations about physiologic thresholds, not one type of cancer has thus far been shown to be controlled by a single gene. This would demand caution in concluding single factor inheritance for cancer in man. Before inbred strains of mice were available and when the only estimate of the genotype was the observation of whether or not the individual mouse developed a tumor, results were obtained which were interpreted as indicating single factor recessive inheritance. Following the development of the inbred strains single factor dominant inheritance was indicated by certain simple crosses. This was true of both pulmonary tumors and mammary gland tumors in mice. The variation between the various inbred strains, however, could not be explained with such simple interpretations. For example, in regard to pulmonary tumors, strain A has an incidence of 90 per cent, strain Swiss 40 per cent, strain BALB/c 20 per cent, strain C3H between 5 and 10 per cent and C57 L and C57 BL less than one per cent. Furthermore, when strain A was outcrossed to three different low-tumor strains the degrees of susceptibility to induced pulmonary tumors observed in the three resultant groups of F_1 hybrids were not the same but were relatively high, medium and low (Heston, 1940). Later by utilizing the number of nodules appearing in the lungs of each individual as a reliable quantitative measure of degree of susceptibility to induced pulmonary tumors and checking these results against those obtained by using latent period as a measure of susceptibility multiple factor inheritance

was established for induced pulmonary tumors (Heston, 1942a). Through hybridization studies multiple factor inheritance has also been established for spontaneous pulmonary tumors (Heston, 1942b). Here, as with the genetic analysis of other spontaneous tumors, there is not a quantitative measure of degree of susceptibility and the problem is more comparable to that of polydactyly in the guinea pig described by Wright, (1934b), i.e. that of a multiple factor character with alternative expression depending upon whether or not the combined action of the genetic and non-genetic factors surpasses a physiologic threshold.

While a quantitative measure of degree of susceptibility to mammary gland tumors in mice has not been available, the vast amount of data on mammary tumors, both from tabulation of incidences in the various strains and from hybridization studies, can be explained only on the basis of multiple factors (Bittner, 1952, Heston, 1945). Burdette (1943) has demonstrated multiple factor inheritance for induced subcutaneous sarcomas, and since the F_1's were intermediate he assumed that at least one factor was dominant and one was recessive. In crosses between high and low leukemic strains Cole and Furth (1941) observed that the incidence of leukemia in the hybrid groups was roughly correlated with the total heredity from the high leukemic strain. Through breeding tests of backcross segregants MacDowell *et al* (1945) established the inheritance of leukemia as multiple factor. Data on other types of tumors in mice consists primarily of incidence data of the inbred strains, but none suggests single factor inheritance.

DOMINANCE

In consideration of multiple factor inheritance of cancer in mice with alternative expression resulting from fluctuation of the combined action of genetic and non-genetic factors about physiologic thresholds, one is reluctant to accept the inheritance of a specific type of cancer in mice or in man as dominant or recessive. The early interpretation of recessive inheritance in mice can probably be explained in that the cancers observed were the result of a relatively low genetic influence together with a strong non-genetic influence. Thus, matings of cancer $\times$ non-cancer seldom yielded cancer whereas cancer $\times$ cancer did. The later interpretation of dominant inheritance may be explained in that in the development of the inbred strains such a high level of genetic influence for specific cancers was concentrated in certain strains that even on outcrossing to low-tumor strains the level was not lowered so much but that a majority of the F_1's developed cancer. The F_1's usually lived longer, giving a longer time for the accumulation of non-genetic influences. Much confusion is avoided by reserving the terms "dominant" and "recessive" to be applied when specific genes have been identified.

"LINKAGE"

Associations between specific genes of the mouse and susceptibility to specific types of tumors indicate the value of similar linkage studies in man and suggest possible physiologic paths through which gene action may influence the probability that a specific type of tumor will occur. Strong (1945) has reported linkage between the brown locus and the stomach tumor induced in his BRS strain, although such linkage was not observed for the spontaneous tumors (Strong and Hollander, 1951). Bittner (1945) reported linkage between brown and mammary gland tumors. In two different experiments (Heston and Deringer, 1948) agouti backcross females from a cross between C3H and C57 BL had a higher incidence of mammary tumors than had the non-agouti females, suggesting an effect of the agouti gene. Little (1934) reported an association between lethal yellow an allele of agouti and mammary gland tumors. The tumors came up earlier in the yellow females than in their non-yellow sibs although the final incidence was lower in the yellow females. Manifestation of the primary action of the gene through the physiology of the endrocrine system was indicated. The yellow females became sexually mature earlier but passed through their reproductive period earlier than did the non-yellow females.

Lethal-yellow increases susceptibility to pulmonary tumors (Heston 1942c, Heston and Deringer 1947), but here the association may be related to the effect of the gene on body size. Association with pulmonary tumors has also been demonstrated for shaker-2, hairless and flexed-tail (Heston, 1941, Heston *et al* 1949, 1951, 1952). It has been established that the association with lethal-yellow was due to the gene *per se*, and the evidence available also indicates that the associations with the other three were probably also owing to the genes *per se* rather than to true linkage. In the case of flexed-tail there appeared to be a closer association with the flexed-tail manifestation of the gene than with the anemia. Although not every factor that affects body weight affects susceptibility, e.g. there is a sex difference in weight but no sex difference in susceptibility, there is a suggestion that these genes may be affecting susceptibility to pulmonary tumors through some effect upon growth in general. Lethal yellow increased susceptibility to pulmonary tumors, whereas the other three genes decreased susceptibility, and lethal yellow increased body weight, whereas the other three decreased body weight.

The dilution gene is similarly of interest. Little *et al*, (1934, 1939) reported an association between dilution and an increased incidence in non-epithelial tumors and MacDowell *et al.* (1945) found that dilution increased the occurrence of leukemia. This gene also has been shown by MacArthur (1949) to increase body size. The relationship of all of these genes to neoplastic and normal growth has important implication in respect to life insurance data reviewed by Tannenbaum (1940) as indicating that obese human beings are

more susceptible to cancer than are individuals of more normal weight. It is suggested that this relationship in man may be basically genetic.

Law (1952b) has established a relationship between flexed tail and leukemia, although there was none between leukemia and the linked genes shaker-2 and waved-2. There was no indication here that the effect of the flexed-tail gene upon the occurrence of leukemia was associated with its effect upon body size. The flexed tailed animals were more susceptible to leukemia than were the non-flexed. In this case the association may be related to the anemia manifestation of the gene in that although the mice recover from the anemia within a few days after birth there may be a weakness in the hematopoietic system which later predisposes to leukemia. This association is particularly interesting in view of the reported higher incidence of pernicious anemia among relatives of leukemia patients than among relatives of control probands (Videbaek, 1947), and adds support to the interpretation of a genetic basis for the relationship in man.

LOCALIZATION OF PRIMARY GENE ACTION

More progress has been made in the study of paths through which unidentified genes influence the development of tumors in the mouse than through which the actions of these identified genes become manifest. Although four specific genes have been shown to influence susceptibility to pulmonary tumors, their effects do not constitute the total genetic control of susceptibility. Strain A and strain C57 L which represent the two extremes in genetic susceptibility do not differ by any of these four genes.

Through transplantation studies (Heston and Dunn, 1951) it has been shown that the primary action of at least most of the genes for susceptibility by which these two strains differ is localized in the pulmonary cells rather than becoming manifest through some general systemic mechanism. Transplants of normal pulmonary tissue from both susceptible strain A and resistant strain L were made in a common host, the F_1 hybrid of these strains. Following the intravenous injection of the carcinogen 1:2:5:6-dibenzanthracene into the host, tumors were frequently found in the strain A transplants but rarely found in the strain C57 L transplants. Thus, the tumor response was controlled by the genotype of the transplant and not by the genotype of the host. In a similar study using the Bagg albino strain and strain DBA, Shapiro and Kirschbaum (1951) obtained comparable results.

Caution should be exercised in transposing these findings in the mouse to man especially since pulmonary cancer in man is bronchiogenic in origin whereas that in the mouse is alveolar in origin (Grady and Stewart, 1940, Mostofi and Larsen, 1951). Furthermore, there have been no studies to show that pulmonary cancer in man is inherited. However, studies on the mouse point to the advisability of genetic studies on pulmonary cancer in man, which,

with the reported increased incidence are more feasible now than several decades ago. If gene action is indicated, and experience with the mouse would suggest that it will be with collection of ample data, this action may be limited to the pulmonary cell in man like it is in the mouse.

Much of the genic action controlling mammary tumor development in mice is not localized in the mammary gland cell (Heston, 1946). By crossing susceptible strain C3H having the milk agent with resistant strain C57 BL without the agent and backcrossing the F_1 females to C57 BL males and to C3H males, two groups of backcross females which differed only in their genotypes were produced (Heston, and Andervont, 1945). Both groups had received the milk agent from their genetically identical mothers yet both groups did not transmit the agent the same. When tested by fostering identical test females the susceptible strain backcross females were able to transmit the agent better than were the resistant strain backcross females as indicated by the higher incidence of tumors in the test females for the susceptible strain backcross. The ability to transmit the agent was influenced by the genotype of each backcross group. This conclusion is supported by the fact that the agent dies out in certain resistant strains (Andervont, 1945). Thus, one of the paths through which genes control whether or not mammary gland tumors will occur in mice is through the control of the propagation and transmission of the milk agent.

Another path through which genes control the occurrence of mammary gland tumors in mice is through gene control over the hormonal stimulation. Crosses between strain A in which virgin females rarely develop mammary tumors and strain C3H in which there is a high incidence in the virgins indicated that genes were acting either through the control of hormonal output or the response of the mammary gland to the hormonal stimulation (Bittner, *et al.* 1944, Heston and Andervont, 1944). Studies of estrus cycles revealed little difference between the strains or their hybrids except that those with a high mammary tumor incidence began their cycles at an earlier age (Deringer, *et al.* 1945). Through transplantation of ovaries, however, Huseby and Bittner (1948) have demonstrated that at least some of the genic control is through control of hormonal production. C3H ovaries in F_1 females resulted in a higher mammary tumor incidence than did A ovaries in F_1 females.

This leaves as a third possible gene action path the control of the response of the mammary gland cell to the hormonal or milk agent stimulation by the genes within the cell. More work is needed to differentiate clearly between this path and either of the other two.

Obviously this whole picture cannot be transposed to man. One cannot expect genes to control breast cancer in human beings through control over some extrachromosomal agent until such an agent has been identified. Furthermore, mice and human beings differ in respect to the role of hormonal stimula-

tion in breast cancer. Women who have had children are reported to have a lower incidence of breast cancer than those who have not had children (Wainwright, 1931), the exact opposite to the situation in mice. What is indicated, however, is that in man as well as in mice the inheritance of breast cancer may be very complex with gene action becoming manifest through a number of paths. Attempts to identify such paths are important for it may be found that certain ones will open the way to the application of control measures.

RELATION BETWEEN GENETIC AND NON-GENETIC FACTORS

Much has been done with mice in revealing the additive relationship between genetic and non-genetic factors and the relative importance of each in the development of tumors. The complex situations encountered are difficult to analyze using human material, but general concepts developed from the mouse are helpful in an attempt to visualize the situation in man.

The malignant change represents some permanent change in the cell. As yet we do not know whether basically this is a change in the cytoplasm i.e., a mutation of a plasmagene or some other component of the cytoplasm, or a change in a gene, i.e., a true mutation in the minds of many geneticists. It resembles a mutation in that it is a chance happening the probability of its occurrence being influenced by the intrinsic and extrinsic factors acting upon the organism or the cell. This happening can be compared with the physiologic threshold described by Wright (1934a, and b) in his analysis of polydactyly and otocephaly in the guinea pig.

The additive effect of the genetic and non-genetic factors has been demonstrated most clearly in respect to pulmonary tumors in mice. With the potent genotype of strain A the malignant change has occurred in the lungs of approximately 90 per cent of the animals of this strain by 18 months of age when kept under usual laboratory conditions. As the length of time for this chance happening to occur is shortened the incidence of tumors is decreased, being approximately 50 per cent at 12 months and 20 per cent at 6 months of age. The change usually occurs in only one focus of the lung giving rise to a single nodule, although there may be two or three but rarely more. Following the intravenous injection of .5 mg. 1,2,5,6-dibenzanthracene, a potent carcinogen, pulmonary tumors occur in 100 per cent of the mice of this strain within 6 weeks and by 4 months the change will have occurred in about 75 foci for there will be on the average 75 nodules. As time increases the number of nodules increases until because of coalescing they no longer can be counted.

Strain C57 L is genetically very resistant to pulmonary tumors. In the history of the strain the malignant change has been observed to occur only 3 times in the lungs of these mice kept under usual laboratory conditions. When strain C57 L mice were injected with the same carcinogen as were the strain A mice the incidence of tumors was raised to only 24 per cent and this incidence

was attained only after keeping the animals for 12 months. Furthermore, most of the animals had but a single nodule.

Strain BALB/c can be cited as one with an intermediate genetic potency. Pulmonary tumors occur in about 20 per cent of the mice of this strain kept under usual laboratory environment. When injected intravenously with .5 mg. dibenzanthracene the malignant change occurred in the lungs of all mice by 5 months but instead of the extremely high number of nodules such as found in strain A mice there was an average of only 10 nodules.

Much other data could be cited to demonstrate further that following the introduction of a carcinogen of uniform dosage, the strains, although all showing an increased pulmonary tumor response, retain their relative positions observed for spontaneous tumors. These carcinogens include a host of compounds including polycyclic hydrocarbons, urethane (Nettleship and Henshaw, 1943) and nitrogen and sulfur mustard (Heston, 1949, 1950). Radiation also increases the occurrence of pulmonary tumors in mice (Lorenz *et al.* 1946). This was first demonstrated with strain A which has a potent genetic influence, but later a significant although less effect was noted in LAF_1 animals which have a less potent genetic influence.

Not all influencing factors, however, add to the pulmonary tumor response. Strain A mice fed a low-calorie diet had fewer tumors than did the controls (Larsen and Heston, 1945).

The concept illustrated here should be kept in mind when attempting to visualize the relative importance of different factors influencing the occurrence of cancer in man. It is to be expected that tumor response of human beings to radiation or other possible industrial agents may likewise be governed in large measure by the genotype of the individual. Because of the great genetic variation of the human population, great variation in response to these possible exogeneous agents may be expected. The inhalation of small amounts of radon by the miners of Schneeberg and Joachimsthal over a long period of time has been suggested as the explanation of the fact that approximately 50 per cent of them died of pulmonary cancer. Before generalizing on the possible effect of radon in causing pulmonary cancer from observations on these isolated communities it is, of course, necessary to investigate the possibility of there having been inbreeding or other patterns of marriages which may have concentrated in these communities genetic factors that may have contributed in large measure to the high incidence of pulmonary cancer.

Mammary gland tumors in mice are inherited in the same general pattern as are pulmonary tumors in that there are multiple genetic and non-genetic factors each of which can influence the probability that the tumor will occur. No factor can be considered as the cause of the mammary tumor but each is to be viewed in respect to its effect upon this probability. This effect can be measured accurately only when all other factors are controlled. The situation

in respect to mammary tumors is more complex than that in respect to pulmonary tumors, for, among the non-genetic factors, there are not only the exogeneous factors such as the chemical carcinogens and radiation, but there are also the endogeneous factors including the milk agent and the hormonal stimulation which are intimately related to the genotype.

The discovery of the extrachromosomal factor in the etiology of mammary cancer in mice by the staff of the Roscoe B. Jackson Memorial Laboratory (1933) and by Korteweg (1934), and the subsequent identification of this factor as a milk agent by Bittner (1936), has had an important bearing upon the cancer problem in man. However, the value of these observations was not as a basis for recommending that mothers not nurse their female babies. Failure of Macklin (1952) in her studies to demonstrate a milk agent in respect to human breast cancer has emphasized the rashness of such a recommendation. The principal value was as a basis for the suggestion of a possible extrachromosomal agent in man and the necessity of work to reveal such an agent if it were present. The dependence of the milk agent upon a suitable genotype has also been of significance in planning investigations with human cancer. It should be emphasized that the agent was revealed through genetic techniques and only after genetically controlled strains were established. Furthermore, the fact that it is transmitted early in the life of the mouse is also of significance. It is to be expected that if there is a virus associated with breast cancer, or any other cancer in man, it may well be revealed through genetic studies in which attention is not only focused upon the individual at the time the cancer occurs, but also throughout his early life and throughout the life of his relatives.

One of the more recent developments in investigations of mammary tumors in mice has been in respect to tumors that occur in the absence of the milk agent (Kirschbaum, 1949, Andervont and Dunn, 1950, Heston, *et al.* 1950). In certain strains the application of carcinogens is required to bring out a large number of these tumors, but in strain $C3H_f$ ($C3H_b$) an incidence of 38 per cent was observed in untreated females. It was necessary, however, that these $C3H_f$ females have many litters in order to have such a high incidence of tumors. The incidence in virgin $C3H_f$ females was less than 5 per cent. A longer latent period was also needed for the tumors to appear. The average tumor age for the $C3H_f$ breeding females was 20 months whereas the tumors occur at an average age of 8 months in the C3H females with the agent.

There was a higher frequency of unusual histologic types among these tumors occurring in the absence of the agent. Tumors with squamous metaplasia classified as adenocanthomas, those with dilated alveolae with muscle elements in the aboundant stroma that Dunn (In Press) has designated as type C, and those with sarcomatous elements classified as carcinosarcomas, occurred relatively frequently in animals without the agent. Tumors arising in females with the agent usually show uniform ascinar structure, i.e., Dunn's type A, or the

cells occur in cords or sheets, i.e., Dunn's type B, whereas the other histologic types occur rarely in the presence of the agent. This variation may, however, be a more direct expression of tumor age rather than presence or absence of the agent.

Biologically these tumors that arise in the absence of the agent may be more comparable with human breast cancer than are those arising with the agent, and a more thorough study of them may reveal further suggestions applicable to human studies. In reciprocal outcrossing of strain $C3H_f$ with low-tumor strain C57 BL (Heston and Deringer, 1952) no maternal influence of any kind was revealed in that there was no difference in tumor incidence of the reciprocal hybrids. This would suggest that the difference between the two parent strains was for the most part genetic, and, since the incidence of tumors in the F_1 groups was intermediate, at least one dominant gene and one recessive gene, or no dominance, was indicated.

These studies do not detract from the importance of the milk agent. One must bear in mind that these $C3H_f$ females, which will have an incidence of mammary tumors of 38 per cent at 20 months in the absence of the milk agent, will have an incidence of 95 per cent at 8 months if they receive the milk agent when nursing. These studies, however, do throw a somewhat different light on the problem of viruses than has often been viewed in the past, and this may be of importance in the human cancer problem. The mammary tumor disease in mice can no longer be looked upon as a virus disease, such as small pox, in which the individual gets the disease only when he gets the virus. It must be considered as a disease the frequency of which can be greatly enhanced by the introduction of a virus, but which may also occur in indistinguishable form in the absence of the virus. The same situation may prevail in man even if a virus is identified in the etiology of human cancer.

SOMATIC MUTATION HYPOTHESIS

Work with the mouse in testing the somatic mutation hypothesis of cancer can have direct bearing upon our understanding of human cancer. If this hypothesis is proved for mice or any other species it should apply to all, including man. Until new techniques are developed and adapted to the detection of gene changes in somatic tissue, support of the hypothesis will have to rest largely upon indirect evidence.

One of the approaches to the problem has been through a search for a positive correlation between carcinogenic and mutagenic potencies of compounds. While the mouse is an excellent animal for carcinogenic studies, it presents certain difficulties in mutagenic studies. The development of the chromosomal map of the mouse, however, is helping to alleviate such difficulties. Strong (1949) has reported mutations in mice induced by subcutaneous injection of 20-methylcholanthrene, one of our most potent carcinogens, and Carr (1948)

also reported mutations in mice induced with the same compound. Unfortunately in both projects the mutations were observed in hybrids, and critical controls were lacking, but both reports are of importance and emphasize the necessity of further testing with controlled stocks. Nitrogen mustard and sulfur mustard which are strong mutagens when tested on Drosophila and other lower organisms (Auerbach, 1949) both have been shown to be potent carcinogens when injected into mice and rats (Boyland and Horning, 1949, Griffin, et al., 1950, Heston, 1949, 1950). On the other hand, mustard oil that Auerbach found to be a weak mutagen, did not increase the occurrence of tumors in a detectable amount. Thus, there appears to be a positive correlation between carcinogenesis and mutagenesis from the work done in mice. It should be pointed out, however, that Burdette (1950) has not found such a correlation in Drosophila. Furthermore, radiation, which when added to nitrogen mustard has an additive effect in inducing mutations in Drosophila, when added to nitrogen mustard in mice has a diminishing effect on carcinogenesis. (Heston, *et al*, unpublished data.)

Other information bearing on this problem has been obtained through dose-response analysis. In an analysis of the number of papillomas arising in mice painted semi-weekly with benzpyrene, Charles and Luce-Clausen (1942) found a straight line relationship when the square-root of the number of papillomas was plotted against time, and expression of dose. This would be expected if the malignant change were the result of two events in the cell, i.e. if the malignant change were the result of a recessive mutation. However, in an analysis of the number of pulmonary tumors occurring in mice injected intravenously with graded doses of dibenzanthracene (Heston and Schneiderman, In Press) a straight line relationship was observed when the number of tumors was plotted against the dose. This result would be expected if the malignant change were dependent upon a single happening in the cell, i.e. a dominant mutation.

While these approaches have not succeeded in proving or disproving the somatic mutation hypothesis they do illustrate how the mouse can be adapted to a search for the answer to this question. When obtained this answer will apply to cancer in man as well as in the mouse. It should be emphasized that proof of the hypothesis would not eliminate any possibility of preventative measures against cancer. The fact remains that there are many factors that increase the probability that this change occurs even though the change may be a mutation. The elimination of each of these factors would decrease to a certain degree the probability of the occurrence of the cancer.

PROBLEMS IN THERAPY

Aside from contributing to our understanding of problems in the development of cancer in man, genetic studies in mice also have been used to clarify problems in the treatment of human cancer. One of the greatest difficulties arising

in the chemotherapy of human cancer, specifically leukemia, has been the development of resistance of the leukemia cells to the drug during the course of treatment. The question arises as to what is the nature of this resistance.

Fortunately the mouse provides an experimental approach to the problem for transplantable mouse leukemias respond to the same chemotherapeutic agents that are effective against human leukemia. Law (1951, 1952a) particularly has studied resistance of transplantable mouse leukemia to folic acid antagonists and has employed techniques similar to those used in the analysis of resistance of bacteria to antibiotics. He has found that through the course of treatment certain strains of leukemia arise which can tolerate the antifolic compounds. Furthermore, some strains were shown later to develop a dependence upon the compounds just as bacteria may develop a dependence upon the antibiotics. These transformations were nonreversible heritable changes. By treating serial leukemic transfers with graduated doses of folic antagonist he demonstrated discrete stepwise increases in resistance. All these observations suggested that the changes were mutations.

The question remained, however, whether or not these changes were independent of the treatment. By adapting the "fluctuation test," utilized by Luria and Delbruck (1943) in studies on bacteria to his study of the leukemia cells, Law was able to demonstrate independence. Starting with a common source of leukemia cells, he established 15 independent sublines and carried each through six transfers. In the seventh transfer, cells of each independent subline but one were inoculated into 10 mice which were then treated with the antifolic compound. The remaining subline was in turn divided into 10 lines with 10 inoculated animals for each line and these were likewise treated. Variation in response of the independent sublines with significantly greater variation among the independent sublines than among the 10 lines formed by dividing the one subline, indicated that these changes had occurred prior to the treatment and were, thus, independent of the treatment. Hence, resistance could be considered as resulting from the selection of mutations occurring independently of the treatment. Basic information such as this is directly applicable to man.

SUMMARY

The following basic facts and concepts derived from studies of the genetics of cancer in mice are probably also applicable to cancer in man.

1. The occurrence of every type of cancer can probably be influenced to some degree by the genotype of the individual.

2. A common genetic basis for two or more types of cancer probably will not conclusively be shown unless common physiologic patterns preceding the occurrence of the different types of cancer can be demonstrated.

3. The expected type of inheritance in cancer is multiple factor inheritance

with alternative expression, the appearance of the cancer depending upon whether or not the combined effect of the genetic and non-genetic factors exceed certain thresholds.

4. Results suggesting dominance in cancer may be owing to fluctuation of the combined effects of genetic and non-genetic factors about physiologic thresholds.

5. Specific genes may influence the occurrence of tumors through various gene action paths. One gene may influence more than one type of tumor through the same or different paths, and one gene may exert the same or opposite effect upon the occurrence of different tumors.

6. Primary gene action may be localized in the cell that becomes malignant or it may become manifest through some general physiologic system such as the endocrine system or through its control of extrachromosomal agents such as the milk agent in mice.

7. Because of additive relationship of genetic and non-genetic factors, the effect of any exogeneous factor on the occurrence of cancer may depend upon the genotype of the individual. Conversely, the influence of the genotype upon the occurrence of cancer may depend upon the non-genetic factors acting upon the individual.

8. Studies on mutagenesis-carcinogenesis correlation and on dose response in the mouse have yielded information supporting the somatic mutation hypothesis. The carcinogenic response in mice of radiation and nitrogen mustard combined, however, does not support the hypothesis nor do certain correlation studies in Drosophila.

9. Resistance of neoplasms to therapy developed during treatment may have a genetic basis.

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