A Reconsideration of the Somatic Mutation Theory of Cancer in the Light of Some Recent Developments

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IN THE STUDY OF ONCOLOGY, one is occasionally induced by obstinate manifestations of cellular anarchy to reconsider what has been, since the time of von Hansemann and Boveri, the much-debated somatic mutation theory of cancer. Since recent experimental investigations have served to renew interest in the theory, one may hope that the theory will be divorced from the *reductio ad absurdum* to which it was once relegated. In a discussion on the nature of gene action, Beadle (*1*) seems to have accounted correctly for the disfavor in which the somatic mutation theory has fallen when he stated that "Possibly one reason why this theory has been looked on with so little favor in certain quarters is that it offers little hope for a cancer cure." It should not be assumed that, if the somatic mutation theory be proved valid, all hope for a cancer cure automatically evaporates.

Recognizing the multiplicity of characters and characteristics that have been shown to be under the control of the genetic constitution of the organism, it is by no means unreasonable to suppose that certain genes also play a prominent role in the direct or indirect initiation and control of cell division in morphogenesis and in reconstitution. The morphology and physiology of a particular organ are the result of the interaction of a multitude of genes. In the development of a structure that is to perform a particular function, there must exist a condition that will assure thousands of cell divisions in predetermined orientation and time schedule to bring about the necessary macroscopic and microscopic differentiation.

Hammett (*2*), who has studied the effect of chemical stimulators on developmental growth, expressed it in these words:

> The ordered production of a species-true organism is the property of heredity. Thus the species specificity in chemical composition, and the superimposed specificity in organ and tissue composition are determined through heredity. Heredity also sets the species, organ, tissue, and cellular specific course of development. Heredity thus selects the characterizing chemical building materials of the developing organism. [Concerning cancer, he continued] . . . the course of development and the distinctive chemical nature of cancer and cancer cells is set by heredity. . . . The fact that cancer cells proliferate true to type, and form other cancer cells through many generations is sufficient evidence for the foregoing dictum.

Cells, whether germinal or somatic, which have been exposed to agencies capable of producing mutations, frequently reflect the effect of the adverse environmental state by giving rise to an anomaly, the persistence of which in the species depends upon the degree of undesirable deviation from the normal, and whether the mutation took place in the germinal or somatic cells.

Many attempts have been made in the past to establish a relationship between the neoplastic cell and chromosomal aberrations. Some have held that there exists a preponderance of aberrant chromosomes in malignant cells. Others have either doubted this or have maintained that such anomalies are not necessarily restricted to the cancer cell. It might also be pointed out that the morphologic alterations observed in *in vitro* cultures of mouse fibroblasts could not be correlated with the sarcomatous transformations of the cells (*3, 4*).

Speculating on the possibility of cancer being the result of a somatic mutation, Morgan and Bridges (*5*) gave a brief résumé of Boveri's cancer theory. Boveri suggested that cancer might result as a consequence of imperfect or irregular division of the chromosomal complex. The abnormal distribution of chromosomes might cause a loss of the factors which normally inhibit the rate of cell growth. However, as pointed out by Morgan and Bridges, such chromosomal aberrations are not necessarily associated with cancer growth. They conceive it to be quite possible that cancer may be due to a recurrent somatic mutation of some gene. In their concluding remarks, the authors feel that it should be kept in view ". . . that what is inherited in cancer may be a gene or complex of genes in which somatic mutation is of sufficient frequency to give the appearance that a gene for cancer is itself inheritable."

Koller (*6*) investigating the cytology of various human tumors, found that the number of chromosomes had a range of 12–48, with a frequency peak at about 30 and another but lower peak at 45 chromosomes. Abnormalities such as stickiness, suppression of spindle, and irregular or polyploid chromosome numbers were in part attributed to a scarcity of food supply and toxic breakdown products.

In cytologic studies of human normal somatic tissues (proliferative stages of the adult uterine epithelium and embryonic tissues), Timonen and Therman (*7*)

and Therman and Timonen (*8*) found that the chromosome number varied considerably, and that the number 48, generally held to be a constant, is *not* the most common outside of the germ line.

Koller (*9*) carried out cytological analysis on some 565 human carcinomas obtained from various organs and tissues and reported that tumors were found in which most of the cell divisions took place through normal mitosis. In some tumors, however, a large proportion of the cells was found to undergo abnormal mitosis. According to Koller, the fundamental cause of increased division rate and malignancy is the excess amount of nucleic acid present in the tumor tissue, and he suggests that it is not improbable that the initial change in nucleic acid metabolism is brought about in the final analysis by a gene mutation which may be assumed to have occurred in the region controlling nucleic acid supply either directly or indirectly.

As the result of his work on tumor transplantability and immunity, Tyzzer (*10*) concludes:

> From the evidence in the biological character of tumors of a permanent modification of somatic tissue, it appears logical to regard a tumor as a manifestation of *somatic mutation.* As a basis for this, there may be modification in the relative value either by loss or addition, or in the nature of factors, any of which, if continuously transmitted thereafter in successive cell generations will constitute a type of mutation. This, unlike the mutations which may affect the germ plasm, is maintained only through artificial transplantation from one individual to another. The tissue of a new growth has thus in certain respects become foreign to the other tissues. Its growth is no longer controlled by the normal inhibiting influences which constitute a regulating mechanism, but it behaves more or less as a parasite living at the expense of its host; and it may excite a reaction of the surrounding tissue which is in some cases more favorable, in other cases less favorable, to its continued growth. Malignant tumors must have feeble antigenic power as well as sufficient resistance to the normal inhibiting influences to provide for continued growth in the animal in which they originate, otherwise reactions sufficient to destroy them would occur more frequently.

Before presenting experimental evidence in support of the somatic mutation theory of cancer it might be well, at least provisionally, to define the theory as follows:

I. A point or regional mutation affecting one or more genes, which directly or indirectly is responsible for the initiation and continuation of an indeterminate number of cell divisions.

II. This type of specific mutation need not necessarily involve a chromosomal aberration or any other kind of visible nuclear change.

III. Cells so mutated may or may not show incomplete differentiation (depending, perhaps, on division rate).

IV. Other mutant characters may be associated with the cell-division factor, the frequency of such occurrence being dependent upon the relative mutagenic susceptibility of other genes. Such an associated mutation may, for example, reveal itself as an alteration in its transplantation pattern, with or without a corresponding change in its antigenic properties.

The process that causes unlimited cell division in cancer is of a different nature than that encountered in ordinary regeneration or wound repair. In the latter case an injury calls forth certain "intercellular wound hormones" capable of inducing cell division. When the repair process is completed the stimulating substances may be said to be depleted, or there follows a restoration of equilibrium between growth stimulators and depressors. Thus the activity of the division factor or gene in wound repair or cell replacement is subservient to the intercellular hormones and therefore fulfills its obligation toward the normal maintenance of the organism. In the case of a neoplasm, however, it appears that an injury of a *specific nature* is required, which in some way either alters (mutates) the division factor so as to give it unrestricted expression, or destroys or mutates a division-inhibiting factor. The division mechanism has lost all restraint in the cancer cell, it flouts (within physiological limits) the systemic regulatory agents. One might say it has acquired an introverted individuality, which it tenaciously retains even though transplanted into relatively compatible hosts through many generations. That the change is of a permanent nature and not influenced by the systemic factors of the host has been demonstrated *in vitro,* where it was found that tumor cells of the mouse (*11*) and of the rat (*12–14*) could be maintained in culture for an indefinite period without losing their malignant characteristics.

There may also be concomitant secondary characteristics in the malignant cell, such as chromosomal aberrations, delimited differentiation, and changes in salt content and in enzymatic and glycolytic activity. These might well be by-products, so to speak, of abnormally proliferating adult tissue cells, or in some particular cases where any one of these factors deviates considerably from the general trend, it may be an associated mutation or response to an environmental change. This may be an audacious supposition, but the fact remains that the one principal character of a neoplasm is unlimited cell division, and that in no case yet observed has it been demonstrated that cell division is the effect, and not the cause, of any of the above-mentioned metabolic and cytologic abnormalities.

The persistent number of cell divisions that characterize a cancer is the one unequivocal feature which, above all, lends credence to the somatic mutation theory. Once abnormal division rate is initiated it continues, the process being an irreversible reaction. Gene reversions probably occur occasionally, as in the germinal cells, but at the most one or a few cells within the mass of malignant tissue may undergo such a change, and, needless to say, such a reversion is of little consequence. Although an exceedingly rare occurrence, spontaneous cancers have been known to regress completely. Woglom (*15*), for example, found that among 2000 mice bearing spontaneous tumors, 13 regressed and 3 fluctuated or remained stationary. This frequency of spontaneous regression (0.8 per cent) is very much higher than that observed in man.

Rohdenburg (*16*) cites Bashford, who estimated that

spontaneous regression in man takes place about once in a hundred thousand, at 0.001 per cent. Even in such a low frequency of regressions, it would appear to be the quintessence of folly to postulate that a gene reversion takes place in such cases which simultaneously affects all the malignant cells composing the tumor. Indeed, regression of a neoplasm requires something other than a reversion to the normal type of cell. It does not necessarily follow that, if cancer is the result of a somatic mutation, no environmental change could be instrumental in bringing about a recession. That regressions, both spontaneous and induced, do occur even in a relatively small percentage of cases, is fortunately the greatest incentive for continued research. Environmental modifications of certain mutant characters are not unknown. For example, in *Drosophila* (*17*), the mutant character *vestigial wing* is a greatly reduced wing size if the flies are raised at one temperature; however, if they are grown at another temperature, the size of the wing approaches that of the normal wild type. A more striking example (*18*), and more pertinent to the problem at hand, is the conditioning effect of the so-called extrachromosomal or milk factor on the percentage of spontaneous mammary cancers in highly inbred mice. In the A strain mice, which are so constituted genetically that about 84 per cent of the females develop spontaneous breast cancers, only 8 per cent develop the cancer if foster-nursed by females of a low breast cancer incidence (C57 black strain).

Mice that have been selectively inbred for many generations to produce a high and low spontaneous tumor incidence have demonstrated that predisposition to cancer, at least in certain tissues, is to a large extent dependent on the genetic constitution.

Man himself furnishes sufficient evidence of cancer predisposition. The high rate of cancer of the uterus and breast in the female, and of the stomach, colon, rectum, and prostate in the male, compared to other organs and tissues is quite significant. The appearance of tumors in human monozygous twins furnishes some rather substantial evidence of genetic predisposition to cancer. In an analysis of tumor development in monozygous and dizygous twins, Macklin (*19*) studied some hundred cases and concluded that tumors appeared in both members of a monozygous twin pair far more frequently than they do in both members of a dizygous twin pair. Tumors of the same type, in the same organ, and occurring at the same time in both members of the pair, were significantly higher in the monozygous than in the dizygous twins.

Phenotypic expression is frequently dependent on certain combinations of interacting or modifying genes. It has been shown, for example, that stable genes can become unstable in the presence of a certain nonallelic gene (*20*). In studying the rate of spontaneous mutations in Drosophila collected from various parts of the world, Demerec (*21*) found that the frequency of spontaneous lethals in the X-chromosome was much higher in a strain from Florida than in other strains. Analysis showed that this higher rate was due to the presence of a recessive gene in the second chromosome. Not only does this gene produce a high frequency of lethals, but it also increases the rate of visible mutations controlled by a number of other genes.

Another illustration of the behavior of an unstable gene is given by Demerec (*22*) in the case of a race of delphiniums. Purple spots on a rose background on the flowers of the rose-variegated delphinium are interpreted as due to changes in the rose gene from rose into its purple allele. Each of these purple spots is the result of a change which took place sometime during the development of the flower. If the change occurs early in development, the cell with the changed gene will divide many times, and therefore produce a large spot; a smaller spot will be produced if the change occurs late in development. The size of the spots therefore indicates the time in ontogeny when the change occurred, and the number of spots is a function of the frequency of changes. From seeds of a self-pollinated variegated plant, a few purple plants are obtained in addition to variegated offspring. These purple plants are the result of a mutation or change of the gene for rose into the gene for purple affecting the germ cells. Similar somatic mutations have been brought about by x-rays in the color of the developing eye of *Drosophila melanogaster* (*23*).

In a discussion on the induction of mutations by carcinogens, Strong (*24*) expressed two aspects of the genetic problem in relation to cancer origin:

> I—susceptibility and resistance to spontaneous, transplanted, and induced tumors—an inherited constitutional state or states in which the germ plasm is definitely involved, and II—the origin of neoplastic lesion by a conversion, somehow or other, from a pre-existing normal somatic tissue—a somatic mutation. The actual process of somatic mutation may either be conditioned or under the control of an inherited or germinal influence, or entirely independent of such intrinsic determination.

Experimental evidence recently accumulated tends to support the generalization of Strong (*24*) that "all mutagens are carcinogens and all carcinogens are mutagens." Thus, in 1945, Strong (*25*) injected mice subcutaneously with methylcholanthrene, and from their untreated descendants obtained 13 mutations involving coat color, thereby showing that germinal mutations could be induced with the carcinogen and at a frequency greater than could be expected by chance alone. In nontreated mice observed over a period of 27 years, the coat color mutation rate was found to be approximately 1 in 26,000. Seven of the induced mutations were repetitions of characters present in the author's stock of mice, and 6 proved to be new ones never observed previous to the methylcholanthrene injection. Two mutants other than coat color (precocious sexual activity and large first litters) were observed in another experiment in which the progenitors were treated with methylcholanthrene (*26*). Strong concluded that ". . . there is evidence the methylcholanthrene has affected the germ plasm by bringing about germinal or point mutations and per-

haps other undetermined effects. It is highly probable, therefore, that methylcholanthrene may also bring about malignancies in tissues by causing mutations to arise in them."

Carr (*27*) produced germinal mutations in mice using a subcutaneous injection of 1 : 2 : 5 : 6 dibenzanthracene. Eighty-three mice, selected from three inbred lines, were treated, and of the thousands raised, no phenotypically detectable spontaneous mutations were observed. Of the 83 mice treated, 7 mutants were found among the F_3 and F_4 offspring, a number far above the expectation where x-rays were used as a source of mutation production. Carr suggested the following argument in support of the observed facts:

> Radiation mutations are almost entirely random, i.e., if a certain gene is mutated by an ionization in one sperm, the chance that the same gene will mutate in another sperm in the same or another individual is not increased. The efficiency of mutation with regard to any given gene is thus almost zero. But this is not necessarily the case with chemicals. If a chemical distributed via the blood stream reacts with a given locus to produce a mutation in one sperm, it is obviously liable to do the same with all other similar loci in other sperms (or ova). An efficiency of mutation at a given locus at 100 per cent can thus be imagined, and then all offspring of an exposed individual will carry the abnormal gene.

In conclusion, Carr states that the types of mutants produced are somewhat different from those produced by high-energy radiations. The hydrocarbons may thus only produce mutations in genes that are less stable than others. This would result in some degree of specificity as required above, suggesting that genes whose unstable nature leads to spontaneous mutation are most readily affected.

Using mustard gas as a mutagen, Auerbach (*28*) found the rate of sex-linked lethals in Drosophila to increase from a normal of 0.2 per cent to over 7 per cent. Mosaics occur in less than 15 per cent in flies treated with x-rays, whereas flies treated with mustard gas produced about 30–50 per cent mosaics. Auerbach's experimental investigations on the various effects of mustard gas on the gene led her to suspect that the treatment does not invariably produce sudden complete mutation, but a tendency to mutate may be acquired which remains latent until a later cell division.

The mutagenicity of carcinogenic compounds was also investigated by Demerec (*29*) who, using aerosolized 1 : 2 : 5 : 6-dibenzanthracene, was able to induce X-chromosome lethals and chromosomal aberrations in Drosophila. The proportion of chromosomal inversions appeared to be higher than obtained in experiments with x-rays. In a later paper (*30*), he showed that of 7 carcinogens tested on Drosophila, 6 were found to be mutagenic. Of 9 noncarcinogens, only two were observed to be mutagens. The available evidence suggested that some chemicals (dibenzanthracene, benzpyrene, and hydroxyazobenzene) induce both gene changes (lethals) and chromosomal aberrations, whereas others (benzanthracene, dimethylaminoazobenzene, and 2-amino-5-azobenzene) are more effective in inducing chromosomal aberrations. Demerec concludes from his experiments that it seems reasonable to infer a common causative mechanism relating mutagenicity and carcinogenicity, and, "Consequently, if cancer originates through a genetic change, our chances of finding ways to prevent it are very, very slight."

That associated changes of a mutational character may occasionally accompany, or at some time follow, a mutation which is specifically concerned with unlimited cell division, has been discovered and critically studied by various workers. Thus experiments on the genetics of tumor transplantability in inbred strains of mice have demonstrated some interesting changes that can occur in the mechanism of tissue compatibility.

A spontaneous carcinoma, designated as the dBrC, arose in the highly inbred dba strain of mice (*31*). Hybrids (F_2) obtained by crossing the dba with Bagg albino (resistant to the dBrC tumor) produced a ratio of 1 susceptible to 4.4 refractory to the tumor. This observed ratio was close to the expected ratio of 1 : 4.61, which indicated the simultaneous presence of 6 dominant factors for the successful transplantation of the tumor. In the course of routine continuation of the tumor in the dba strain, one of the transplants was found to grow with unusual rapidity. Subtransplants of this rapidly growing tumor showed a continuation of this new characteristic. This "new" tumor, called the dBrCX by Strong, was found to grow in all F_1's and F_2's and in all the original dba strain. It also grew in the original nonsusceptible stock, and in other mice irrespective of their genetic relationships. Thus, from a state of high specificity (involving 6 factors), it developed into a tumor completely nonspecific (1 factor), and at the same time showing an increased proliferative vigor. Careful examination in subsequent transplants of the dBrCX revealed another difference in growth between two tumors which thereafter received the terms dBrCm and dBrCsp. The dBrCm gave a 9 : 7 ratio in F_2, and the dBrCsp showed a 3 : 1 ratio in the F_2.

Strong concluded from the behavior of this tumor that a somatic mutation (the term being used in the broadest sense) can occur within the malignant cell which changes its reaction potential and other physiological activities. He suggested that the nature of the mutational process ". . . may either be a change or shifting of a complete chromosome or chromosomes, or a change or changes within a chromosome or chromosomes (genic), or it may be even cytoplasmic in nature." Such mutational changes as observed by Strong have also been found by Bittner (*32*) and by Cloudman (*33*). The interesting aspect is that in every case the change has been from a condition of high specificity to one of low or no specificity; in other words, from a multiple toward a single-factor condition. As Little (*34*) points out, these sudden changes are properly definable as mutations; however, before

they can be established as "gene" mutations, it will be necessary to find a means for identifying the genes involved. Obviously, if such genes could be identified it would place the somatic mutation theory of cancer on a firm foundation, but the important fact remains that the abrupt changes are self-perpetuating.

In view of the experimental evidence collected in recent years, it may be concluded with some degree of confidence that the somatic mutation theory of cancer does not oppose the facts that have so far been brought to light. Undoubtedly there remains much research to be done before the theory can be either proved or disproved. An analysis of the problem at least makes tenable, for the present, the proposal that the change from the normal to the malignant cell is of the nature of somatic mutation, be it a nuclear or a cytoplasmic change, directly or indirectly involving the division mechanism.

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News and Notes

Scientists in the News

Wade Arnold, executive producer of the National Broadcasting Company, was named as first winner of the American Heart Association's annual Howard W. Blakeslee Award for outstanding scientific reporting on heart and blood vessel diseases. Mr. Arnold was cited for writing and producing "Only One to a Customer," a documentary radio program broadcast last year.

J. Leroy Bennett, manager of chemical operations for the Explosives Department of Hercules Powder Company since 1931, has retired after 46 years of service with the company.

Osborne Bezanson, chemist, and president of the Chemstrand Corporation. has been named chairman of the board. He will be succeeded as president by **Henry H. Bitler,** now of American Viscose Corporation, the appointments becoming effective Dec. 1.

M. R. Clarkson, deputy administrator of the Agricultural Research Administration, has been placed in charge of the Department of Agriculture's program for eradication of vesicular exanthema, a disease of hogs.

President Howard L. Bevis, of the Ohio State University, on April 17 recommended to the University's Board of Trustees that **Byron T. Darling,** associate professor of physics, be dismissed from the University faculty, effective as of that date.

The recommendation was made after a hearing given Professor Darling and attended by the members of the University faculty, the members of the president's office, Professor Darling, Joseph Forer, his counsel, and James C. Harris, assistant professor, Department of Physics.

Dr. Darling's refusal, on the grounds of his rights under the Fifth Amendment, to answer questions put to him by the House Un-American Activities Committee in Washington, March 13, as to whether he then belonged or ever had belonged to the Communist party or any related organization, and whether he had ever performed services for or received funds from that party or such organizations "did grave injury to the University and its faculty," to quote the president. "By refusing to say whether certain of his colleagues were Communists, he cast an unwarranted aspersion upon them individually.

"These considerations lead only to the conclusion that Dr. Darling has shown his unfitness for the position he holds. They show a lack of candor and moral integrity in matters vital to his professorial status. They show gross insubordination to University policy. They show conduct clearly inimical to the best interests of the University."

The University president said that Dr. Darling on the Ohio State campus and throughout the country