of a simple type, and many spark chambers. For most of the inelastic colliding-beam interactions, the momentum of every charged particle coming out would be measured to within 0.25 percent and the flight time would identify the particle as a proton, a π-meson or a K-meson. The circulating currents and interaction rates would be comparable to those of experiment 1, but only the collisions that produced large numbers of decay particles would be recorded. The enormous amount of information obtained from this experiment would have to be analyzed by computer, as is customary for bubble-chamber experiments at the energy now obtainable.

3) *Search for the intermediate boson.* At present there is considerable excitement among high-energy physicists about the existence of a particular new particle. It is a shortlived resonance, if it exists at all. If it is found, it will help explain the field of weak interactions, to which the recent Columbia-Brookhaven neutrino experiment and the parity-nonconservation experiments belong. Perhaps the intermediate boson will be found with existing accelerators. If not, it will only be because the mass of the boson is too great for it to be produced at the energies now available. In that case, it should be found in the experiment of Fig. 10, through decay of the boson into an energetic, penetrating μ-meson. The heart of the apparatus is a magnetized iron shield, which will attenuate all charged particles except μ-mesons and permit rough measurement of the energy of the particles which pass through. This experiment, if still necessary when the first proton storage ring is completed, will require a circulating current of 5 to 20 amperes per ring. It appears that such currents can be stored and will survive for several hours. At a pressure of 10^{-10} mm-Hg, the background rate in this experiment would be one interaction per 5 microseconds. This is low enough for rejection by the electronic circuits to be possible.

At present there are only a few of us who are actively working on storage-ring experiments. We enjoy being in a new and exciting field, and we hope that this technique will be strengthened by ideas from many more physicists.

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Biological Mechanisms Underlying the Aging Process

The ideas and techniques of genetics are being used to obtain new insight into the problems of aging.

Howard J. Curtis

The phenomenon of aging is one with which every child is familiar. Everyone realizes that he will undergo adverse changes, with the passage of time, which will eventually lead to death in one form or another, and accepts this as inevitable. It is difficult to think of a biological process of more interest to most adults, and yet through the years the explanations for this phenomenon have mostly been couched in vague generalities. Even today gerontologists cannot agree upon a definition of aging.

It would be quite impossible in one brief article to cover the vast literature on the subject or discuss even a fraction of the theories of aging. However, an attempt will be made to present recent ideas and experiments on this subject in the light of modern biological thought.

Clearly, aging is not merely something which leads to death, for acceptance of this idea would lead to such absurd conclusions as, for example, that automobiles are causing aging in the American population because they are decreasing the life expectancy. In this context, diseases and even cancer might be put in the same category as the automobile, and the question then arises, What is left? Certainly the organism continually "runs down," and which disease finally causes death is often merely a matter of chance. The phrase "died of old age" is no longer in vogue, but the idea behind it is still pertinent. On this basis it seems reasonable to define aging, as Comfort does (*1*), as a biological process which causes increased susceptibility to disease. There are some obvious exceptions, but as a generalization this definition seems to stand up reasonably well. Even cancer and atherosclerosis would then be considered biological phenomena separate from the phenomenon of aging. Thus, senescent tissue provides a favorable environment for some diseases, such as cancer, and withstands the stress of other diseases less well than younger tissues do.

On this basis, then, one must ask what the nature of senescent tissue is and what causes the change from young to old tissue. The many theories which have been put forward to account for aging have been discussed in a number of recent publications (*1*, *2*) and are dealt with only briefly here, under three general categories.

First is the group of theories which postulates the accumulation of deleterious products of metabolism as a cause of aging. Certainly products such as collagen accumulate in some tissues and give these organs the appearance of old organs. The skin is a familiar example. Accumulation occurs in some tissues very markedly and in others practically not at all. Further, organs like skeletal muscle which show little, if any, ac-

The author is chairman of the biology department at Brookhaven National Laboratory, Upton, N.Y.

cumulation of products show marked aging changes; changes in muscular ability are among the first signs of aging. At this point it would be premature to dismiss this theory as untenable, but it does seem more likely that these products accumulate in certain organs as a result of aging than that the accumulations are its cause.

Next are theories which Comfort (*1*) has classed as "wear and tear" theories, which postulate that each stress to which an organism is subjected takes its toll, and that the organism finally wears out. Pearl's "rate of living" theory, and a number of others, come in this category. According to this view, the cells of an organ are endowed with a certain complement of enzymes, for example, at the time the organ is formed, and when this complement is used up, the organ can no longer function properly. This concept deserves serious thought and is discussed in detail later.

The third view is the mutation theory, according to which the somatic cells of the body gradually accumulate deleterious genes by mutation, which cause the cells, and thus the organism, to function less efficiently. This theory has the advantage of being a very definite concept but the disadvantage of being very difficult to prove or disprove experimentally. This, too, is discussed in detail later.

With the discovery that radiation can apparently accelerate the aging process in animals (Fig. 1), a new avenue to the study of aging was opened (*3*). It allowed one to formulate theories of aging in specific terms and to test them experimentally. Over the past 10 years much progress has been made, and we are now in a position to accept or reject theories of aging on the basis of definite experimental evidence. It is the purpose of this article to consider in more detail the wear-and-tear theory and the somatic mutation theory, and to present a modified concept of the aging phenomenon which has arisen from these considerations.

Wear-and-Tear Theory of Aging

The fact that all inanimate objects wear out makes it seem quite natural to think of aging in the same terms. The idea that a disease causes a shortening of the life span, even though there may be apparent complete recovery, is a very old one. However, direct verification of the idea has been lacking. Jones (*4*) and Comfort (*1*) have accumulated a great many actuarial data indicating that, with increase in the stress (usually disease) to which a population of men or animals is subjected, the life expectancy decreases and thus, presumably, aging is accelerated. However, they define aging as an increase in the age-specific death rate, and it is not clear whether the stress is accelerating the aging (as defined above) or whether the populations are not living as long because the probability of an individual's receiving a fatal stress is increased. Certainly the automobile has increased the age-specific death rate of the average American but has not accelerated his aging. Selye and Prioreschi (*5*) have repeatedly emphasized the importance of stress in aging, but unfortunately without supporting experimental data.

Thus, while it seems obvious that old animals are less able to withstand stress than young animals, no information has been available indicating whether or not stress per se causes aging. It has been widely argued that the reason radiation seems to accelerate aging is that it acts as a nonspecific stress.

A few years ago a series of experiments was undertaken, designed to test experimentally the wear-and-tear theory of aging and to assess the degree to which radiation may be considered a unique stress (*6*). The result of one such experiment is shown in Fig. 2. Here, mice were subjected to one of two generalized stresses: nitrogen mustard and typhoid toxoid were administered in such doses that about half the animals died within a day. The survivors were allowed to live out their lives, and their survival curves were compared with those of similar animals that had received a mid-lethal dose of x-rays, and with those of controls. As may be seen in Fig. 2, the animals that had received the chemical stress had as good a life expectancy as the controls, but the ones that had received a comparable dose of radiation had a markedly reduced life expectancy.

This experiment failed to provide support for the view that stress is a factor in aging. In order to test the theory further, experiments with mice were undertaken in which other kinds

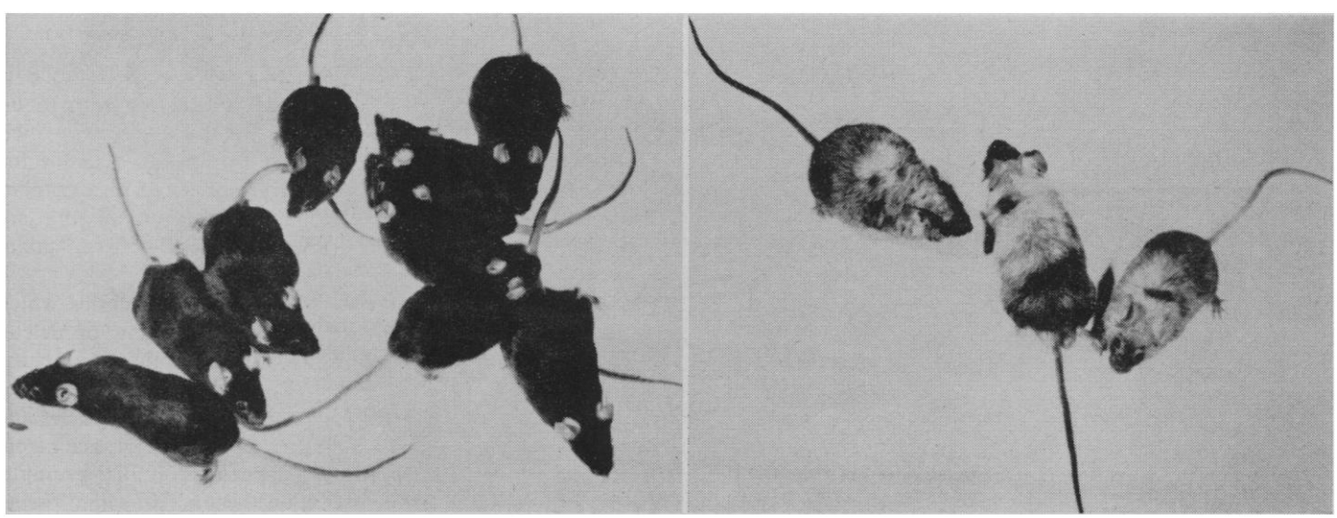

Fig. 1. Two groups of 14-month-old mice which were originally identical. The group at left was untreated; the group at right received a large but nonfatal dose of radiation as young adults. There are only three surviving members of the treated group, and they are grey and senile, while mice in the untreated group are all normal, healthy, and active. [Curtis (*29*)]

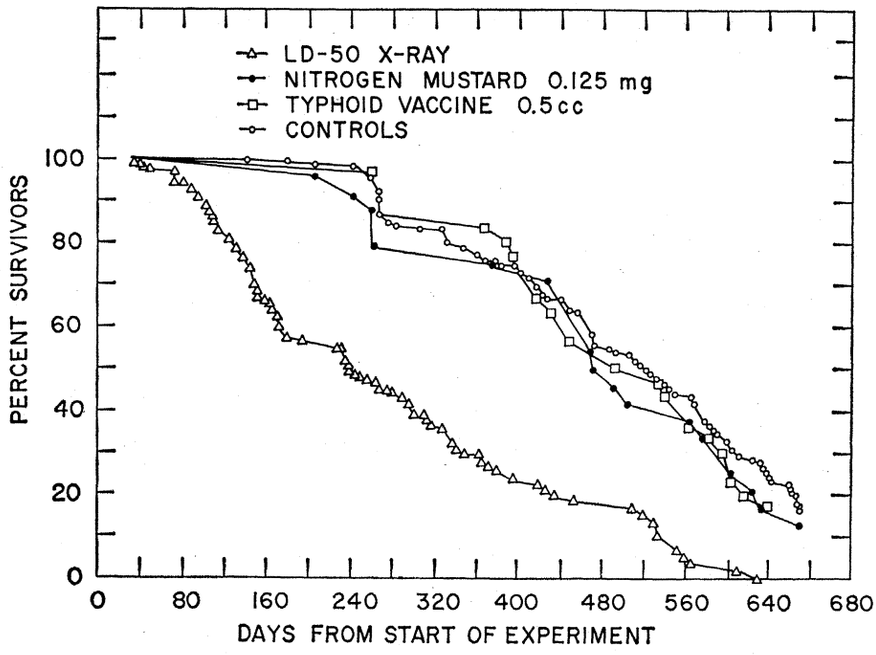

Fig. 2. Survival curves for mice given the treatment indicated when 2 months old. The curves begin 30 days after treatment, to eliminate acute mortality from the record. They show that single massive but nonlethal doses of noxious chemicals do not decrease life expectancy, whereas single massive but nonlethal doses of x-rays do so markedly.

of stress were used—stresses which could be extended over a period comparable to the life span of the animal, so that the effect of the accumulation of stresses might be evaluated (*6*). A number of nonspecific, chemical stress agents were used. These included nitrogen mustard, administered both intravenously and intraperitoneally; tetanus toxoid; tetanus toxin; typhoid toxoid; and turpentine injected subcutaneously, which caused large sterile ulcers to form. All these agents were administered as often as was possible without killing the animals. In some cases this meant administering an almost lethal dose of the substance three times a week for two-thirds of the life span of the mice. One such experiment is represented in Fig. 3; as may be seen, after this severe treatment the life expectancy of the mice was unchanged.

These experiments make it seem highly unlikely that a nonspecific stress per se is involved in the aging process. However, it cannot be denied that a specific stress which severely damages one organ may cause a shortening of the life span. A case in point is mercury poisoning which causes damage to the kidney. Experiments with mice have shown that even when the kidney is severely damaged, as indicated by kidney-function tests, it may be many months before the animal dies somewhat prematurely (*7*). Here the mercury has produced a "weak link," and the normal aging process progresses to the point where the kidney finally fails. Thus, here again stress seems not to be a basic factor in the aging process.

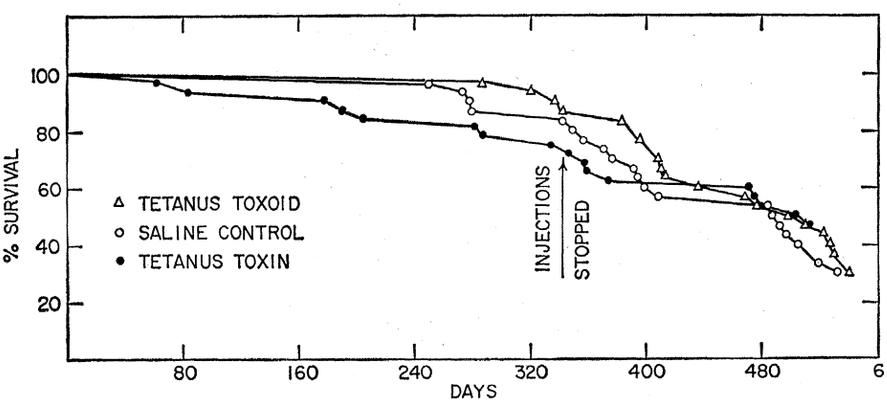

Fig. 3. Survival curves for mice that received massive but nonlethal doses of noxious chemicals weekly for a large part of their lives. Even such drastic treatment did not affect their life expectancy. [Curtis and Gebhard (*30*)]

Thus, the mammalian organism seems to be constructed in such a way that it can deal with most stress situations and emerge unharmed. There seem to be relatively few stresses from which the body cannot recover completely. When the stress has irreparably damaged an organ, some other process seems inevitably to move forward to add to the damage, and it is this other, elusive entity which we refer to as aging. In addition, the experiments pointed to the uniqueness of radiation as a stress agent and emphasized the similarities of radiation-induced aging and natural aging.

One continually hears that the life span of the average American has been dramatically increased in recent years through advances in medical science, and this is perfectly true. However, the maximum life span of man has apparently not changed since biblical times, and all modern medicine has done is to allow a larger fraction of the population to have a life span close to the maximum.

The Somatic Mutation Theory

The idea behind the somatic mutation theory is quite old, but only recently has it been formulated in definite terms. Failla (*8*), Curtis (*9*), Szilard (*10*), and others have pointed out the possible deleterious effects which might build up if spontaneous mutations accumulated in both the dividing and the nondividing somatic cells. Certainly the vast majority of mutations must be deleterious, so if the organs of older animals contain appreciable numbers of cells which are carrying mutations, it is a virtual certainty that the organs are functioning less efficiently than they otherwise would. Even in cells which do not undergo division in the adult, damage may accumulate in the cell nucleus, which is ultimately called upon to exercise a regulatory function that cannot be properly carried out with damaged nuclear material.

As attractive as this theory is, direct

evidence pertaining to it has been very fragmentary until very recently. The fact that radiation seems to accelerate the aging process, and is also one of the most potent mutagenic agents known, is very strong evidence in support of the theory. Szilard has produced a mathematical model of the aging process based on the somatic mutation idea, and has shown not only the plausibility of the idea but the degree to which various factors, including radiation, which alter the genetic structure of the somatic cells may be expected to alter the life span of populations.

The techniques which have been developed recently for viewing chromosomes in the human being have been applied to this problem. Bender and Gooch (*11*) have shown that blood cells from individuals accidentally exposed to even very moderate doses of radiation have abnormal chromosomes even several years after the exposure. Further, Jacobs *et al.* (*12*) have shown that in human blood cells the number of cells having abnormal numbers of chromosomes increases with age. The origin of these abnormalities is uncertain, since infants seem to exhibit them to a surprising degree, but there seems no doubt that the chromosomes of these blood cells are changing with age.

The problem in this theory is that of developing a quantitative measure of mutations in somatic cells. This has never been accomplished directly, but over the past few years an indirect method has been developed at Brookhaven National Laboratory for scoring the numbers of chromosome abnormalities in liver cells of mice, and this quantity is assumed proportional to the mutation rate in these cells. The rationale for the method is derived from work on plants, where somatic cells differentiate into germinal cells, so that it is possible to score true mutations in somatic cells. At the same time the chromosome aberrations in these somatic cells may be scored by observing the cells in the process of cell division and noting the percentage which show abnormal mitoses. This percentage can be related to the percentage of true mutations found in the next generation. In all cases (see *13*) it has been found that the frequency of aberration is directly proportional to the frequency of mutation. If one assumes that the same criteria can be applied to the somatic cells of animals, then it should be possible to look at the chromosomes of animal cells and judge the degree of damage; this would give an index of the numbers of mutations present in these cells.

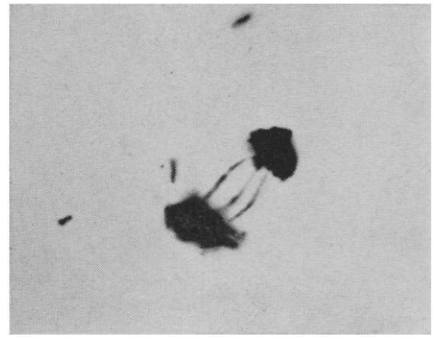

Fig. 4. Photomicrograph of an abnormal dividing liver cell. The two daughter nuclei are still joined by three chromosome strands, which will eventually break, and there are three pieces of chromosomes which did not take part in the mitosis. In a normal mitosis all chromosomes pull apart cleanly. [Curtis (*29*)]

Two difficulties present themselves immediately. (i) It is necessary to observe a cell in the process of cell division in order to judge whether mitosis is normal, and the vast majority of all cells in the adult animal undergo cell division rarely, if at all. (ii) The logical cells to observe, the cells of the bone marrow or the intestinal epithelium, divide so rapidly that most of the chromosome aberrations are obliterated by the repeated cell divisions. The aberrations which survive are mostly chromosome deletions or rearrangements which must be scored at metaphase, by extremely painstaking techniques. If the chromosome damage is of such a nature that the cell cannot complete a cell division, that cell line will be lost and that mutation will not be scored.

However, there are a few tissues in the body which have cells that very rarely divide spontaneously but that can be made to divide if given the proper stimulus. The liver is a good example of such an organ. If a liver cell acquires a lesion in the genome which will eventually lead to an irregular cell division, it stores this until it receives a stimulus to divide, and at that time the aberration can be observed under the microscope.

The technique which is used at this laboratory for studies on mice is based on the work of Albert (*14*) and others. It consists of giving the mouse, subcutaneously, a dose of carbon tetrachloride sufficient to destroy about 65 percent of the liver. Regeneration starts soon, and the rate of mitosis reaches a peak at 72 hours, at which time the animal is killed and a small bit of liver tissue is fixed in formalin. The tissue is then minced until individual cells, after staining, can be "squashed" on a microscope slide. They are examined, and all cells in the later stages of mitosis are carefully examined and scored as either normal or abnormal. The abnormalities usually are either "bridges" (this means that the chromosomes have not separated cleanly or evenly in mitosis) or fragments of chromosomes which do not take part in the mitosis (Fig. 4). If either of these conditions is observed, this certainly indicates the presence of a mutation. On the other hand, many mutations could be missed in this method of scoring. The numbers, then, represent minimum numbers of aberrant cells.

With this method of estimating mutations, an experimental program was undertaken to test the validity of the mutation theory of aging. First, the frequency of chromosome aberration was scored as a function of age (*15*). The results of one such experiment are shown in Fig. 5. It may be seen that in this experiment the aberrations increased steadily and reached a value of 22 percent when the mice were only 12 months old. Since this is a minimum value, one can speculate that at this time most, if not all, of the cells of the liver were carrying mutations. Second, since radiation mimics the aging process, the aberrations were scored as a function of time after x-irradiation (*15*). The results of this experiment are also shown in Fig. 5, and it may be seen that the frequency of aberration increases immediately and then declines very slowly over a period of months. Thus radiation, the only known agent which accelerates aging, also increases the number of mutations, as measured by this method.

Radiation is not the only mutagenic agent, so one would reason that other mutagenic agents should also cause aging. Consequently, the mutagen nitrogen mustard was administered to animals in just sublethal doses, to test the effect on life span. No effect on life span was found. Even when the agent was administered as often as three times a week for over two-thirds of the normal life span of the animals, no change in life span was observed (*15*). It is known that this agent breaks chromosomes in bone marrow and in-

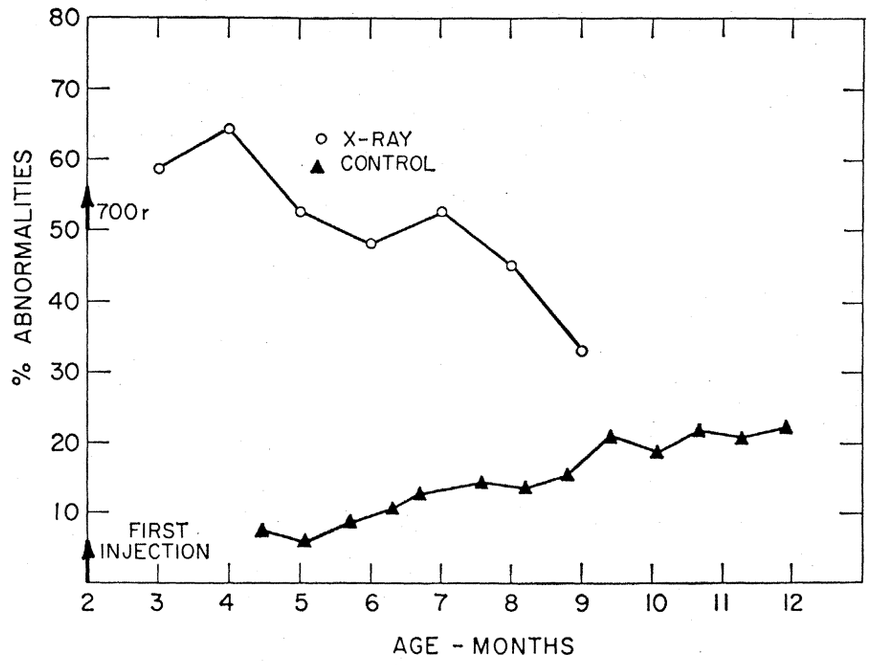

Fig. 5. Chromosome aberrations in liver cells as a function of age in normal mice and in mice that had received a large dose of x-rays. The curves show the steady increase in the number of chromosome aberrations (mutations) with normal aging and the dramatic increase and slow return toward normal in irradiated mice. [Stevenson and Curtis (*15*)]

testinal cells, but these are cells which undergo division quite rapidly. To test whether this agent caused chromosomal damage in other types of somatic cells, aberrations were scored, by the method described, in liver cells during long-term administration of large doses of the drug (*15*). It was found that there was no significant increase in the frequency of aberration in the experimental animals over the frequency in the controls. Here again the theory is supported in a negative way, since an agent which does not cause chromosome damage to cells which rarely undergo cell division (the vast majority of all the cells in the body) does not cause aging. More recent work by Conklin *et al.* (*16*) and by Alexander and Connell (*17*) has shown that some of the other chemical mutagens do cause some shortening of the life span, but unfortunately it is not known whether they also cause chromosome aberrations in liver cells.

It has been known for many years that administration of low-level x- or gamma radiation over a period of months is only about a quarter as effective in shortening life as a single administration of high-level x- or gamma radiation. In a recent series of experiments (*18*) (Fig. 6) it was found that chromosome aberrations develop rather slowly in mice subjected to long-term, low-level gamma irradiation; indeed, gamma radiation administered in this way is only about a quarter as effective in producing abberations as gamma radiation given in single large doses. On the other hand, long-term, low-level neutron irradiation is just as effective in shortening life as large single doses of neutron radiation. In recent experiments (*19*) (Fig. 7) it has been shown that chromosome aberrations develop very rapidly during long-term, low-level neutron irradiation, and that neutron radiation administered in this way is just as effective as neutron radiation given in single large doses. These two sets of experiments give a sound cytological explanation of the known differences in life-shortening effect between x-rays and neutrons and at the same time lend considerable support to the mutation theory of aging. These experiments also show that a good deal of chromosomal recovery can take place after low-level x-irradiation but that none can occur after low-level neutron irradiation.

It is well known that some inbred strains of mice are long-lived and some are short-lived. From recent experiments in this laboratory (*20*), data have been obtained on chromosome aberrations in normal females of a long-lived strain (C57BL/6J) and a short-lived strain (A/HEJ) obtained from the Jackson Memorial Laboratory. The median life span of the former is 600 days and of the latter, 395 days (*21*). The results of this experiment are shown in Fig. 8. As may be seen, in these two strains the rather dramatic difference in life span is correlated with equally dramatic differences in the development of chromosome aberrations. Whereas this is the only experiment specifically designed to measure differences in chromosome aberration in strains of mice having different life expectancies, data obtained on normal mice from other strains for different purposes are quite consistent with these findings. Mice of the two other strains used had life expectancies between those of strains C57BL/6J and A/HEJ and developed chromosome aberrations at rates between the rates for these two strains.

Whereas there is much yet to be done, the correlations observed so far between the development of chromosome aberrations (and presumably somatic mutations) and life span are very impressive. In all instances so far investigated there is a qualitative relation between the development of aberrations and the life span, and in some cases there is a reasonably good quantitative correlation. Factors, either spontaneous or artificial, which increase the mutation rate also decrease the life span. In view of this, it would be very surprising if there were not a casual relationship between them.

Modification of the Simple Theory

The simple mutation theory postulates that when the somatic cells of the body accumulate a certain number of mutations, senescence and death follow. The experiments at the Brookhaven Laboratory clearly indicate that this theory must be modified in two ways.

First, whereas the mutagen nitrogen mustard did not shorten life or cause chromosome breaks in liver cells, it did cause breaks in cells of the hematopoietic system, in gastric mucosa, and presumably in other cells of the body which undergo rather rapid cell division. From this it seems reasonable to conclude that organs containing cells which divide rapidly do not take part in the aging process. When cells divide they tend to throw off deleterious mutations in the course of a few divisions. Cells carrying mutations are defective and cannot survive the competition of normal cells. The evidence from plants shows this especially clearly. On this basis, then, it appears that only organs

in which the cells do not undergo division, or in which they rarely do so, take part in the process of senescence.

Secondly, young animals given large single doses of radiation immediately develop a high percentage of aberrations, a much higher percentage in fact than the normal mice develop when they are very old and senile. Yet these young irradiated mice are healthy and vigorous and will not become senile for many months, although they will become so before the nonirradiated controls do. At first glance this seems to directly contradict the theory, but in the light of recent evidence concerning the role of the nucleus in cellular function, the theory can be modified in such a way as to be strengthened by this finding. Before I discuss this directly, it is necessary to digress briefly to outline this evidence and the resulting concepts.

The Role of DNA in Cell Function

Current ideas on the role of the nucleic acids in cell function are too complex to be discussed in detail here. In brief, it is now felt that the information necessary for the complete functioning of the living cell is contained within the deoxyribonucleic acids (DNA) that constitute the chromosomes of the cell, which reside in the cell nucleus. In the simplest concept there is a single DNA molecule responsible for each function which the cell must perform, and each one is different from all the others. All true growth is by cell division; at the time of cell division each one of these molecules replicates itself, and one goes to each daughter cell as part of the chromosome structure of the nucleus.

The DNA molecules regulate the function of the cell through complex reactions involving an intermediate set of molecules, the ribonucleic acids (RNA). Each DNA molecule presumably can synthesize its corresponding RNA molecule, which then diffuses out into the cytoplasm of the cell where it is responsible for the synthesis of a particular enzyme. It is the amount and kind of enzymes which determine the function of the cell.

The life of an animal starts from a single cell, and as the embryo develops, cells become specialized—liver cells, kidney cells, and so on—to perform different functions. This process is known as differentiation and is presumably brought about by the accentuation of certain cell functions and the suppression of others. Thus, all cells in the body are thought to be potentially capable of performing the function of all other cells. This means that some highly differentiated cells, such as the nerve cells of the brain, have a very great many DNA molecules in their nuclei which are never used.

For each functional DNA molecule there are, at any one time, a great many corresponding RNA molecules and even more corresponding protein molecules. Presumably there are feedback mechanisms which regulate these synthetic processes to keep a dynamic equilibrium. An outside force which does not completely disrupt the cell may cause a momentary perturbation, but this can be corrected for by these regulatory mechanisms. This is true unless the damage is such that an essential DNA molecule is irreparably damaged. In that case a vital cellular function would be eliminated, and as the cell gradually became depleted of the corresponding RNA and protein molecules, it would die. This is a molecular description of a lethal mutation, and mutagenic agents are ones which have the ability to destroy DNA molecules within the nucleus without destroying the cell. A nonlethal mutation is one which changes a DNA molecule in such a way that the cell performs a somewhat different function but does not die.

Certainly the most difficult task a living cell ever performs is that of cell division. It is easy to think of a cell living normally after a great many of its nonessential DNA molecules have been destroyed. However, if this cell were called upon to undergo cell division, it would be quite incapable of doing so and would die in the attempt. This phenomenon has often been observed after irradiation. The irradiated cell appears normal and indeed functions normally until the time comes for it to undergo cell division, and then it usually either dies or produces bizarre daughter cells. This is the phenomenon which underlies most reactions of living matter to moderate doses of radiation.

DNA and Aging

With this background it is interesting to look at the mutation theory of aging. It is known that spontaneous mutations occur in all cells, and one is tempted

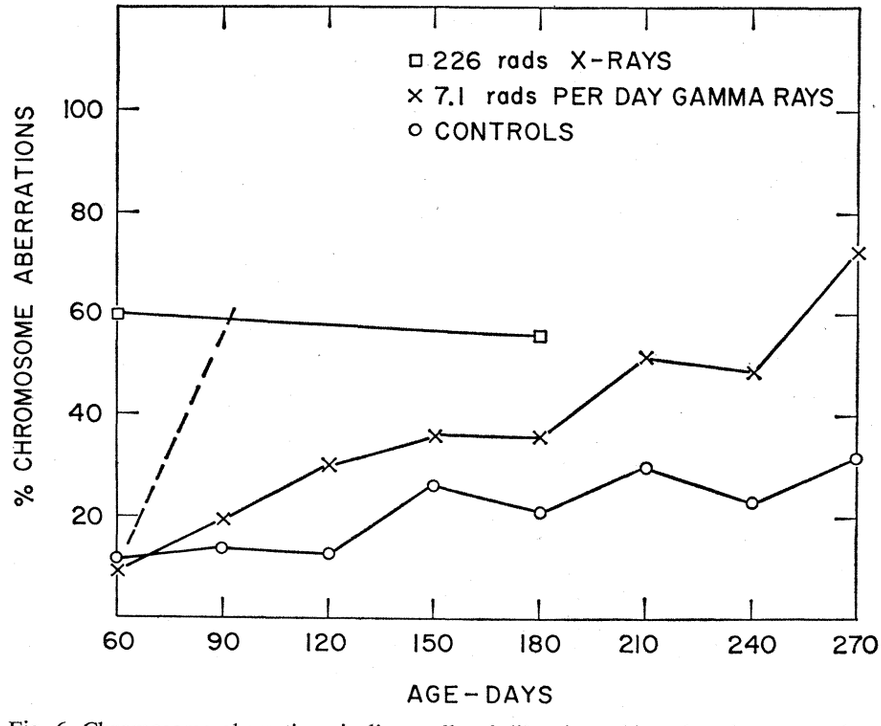

Fig. 6. Chromosome aberrations in liver cells of (i) mice subjected to long-term, low-level gamma radiation, (ii) mice given a single large dose of x-irradiation, and (iii) controls. The dashed curve shows the rate of build-up which would be expected for the mice of group i if long-term, low-level irradiation were as effective in causing chromosome damage as a single large dose is. Since the experimental curve for the mice of group i is very different from the expected curve, it must be concluded that chromosome healing takes place after gamma irradiation at low doses. [Curtis and Crowley (*18*)]

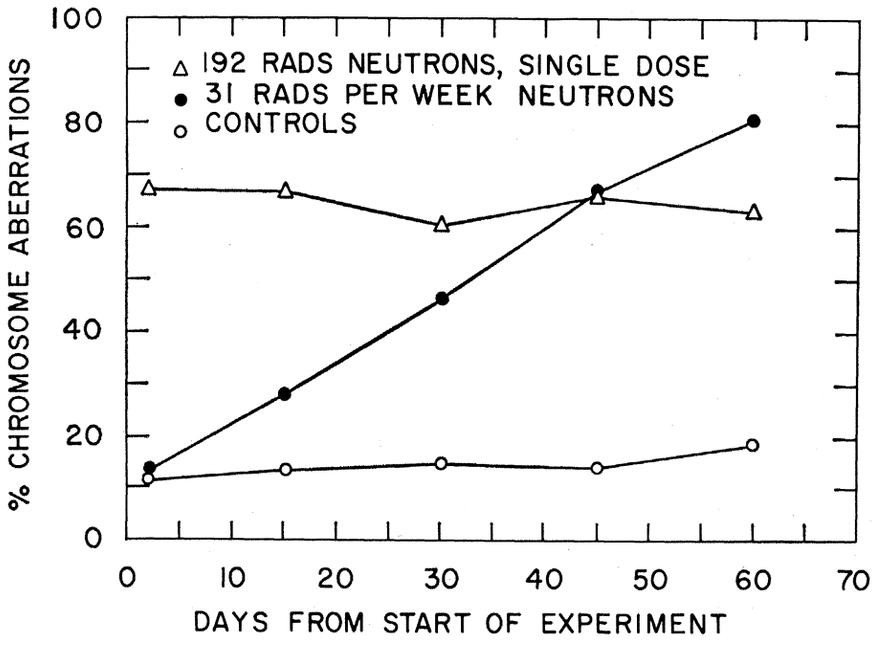

Fig. 7. Chromosome aberrations in liver cells of (i) mice subjected to long-term, low-level neutron radiation, (ii) mice given a single large dose of neutron irradiation, and (iii) controls. If the low-level and the high-level neutron irradiation are equally effective in causing chromosome aberrations, the percentage of aberrations will be the same for groups i and ii at 43 days, and it may be seen that such is the case. These data also show that there is no chromosome healing after neutron irradiation even at very small doses. [Curtis and Tilley (*19*)]

to speculate that as an animal accumulates these mutations the various cells of the body become less efficient in performing their functions, since practically all mutations are deleterious.

One can divide the cells of the body broadly into three classes: those which regularly undergo cell division, such as cells of the skin; those which rarely divide, such as cells of the liver; and those which practically never divide in adult life, such as brain cells. If a mutation occurs in a cell which divides often, it will probably cause little damage to the organ of which it is a part, because the cell will either die directly or lose out in the struggle for existence in competition with its normal neighbors. On the other hand, if a mutation (DNA damage) occurs in a nondividing cell, the organ of which that cell is a part will suffer, because that defective cell will be retained indefinitely. Cells which seldom divide would play an intermediate role in this process.

On this basis, the organs with nondividing cells should be the ones to cause senescence, while the ones with rapidly dividing cells should be essentially immortal, provided they are well supported. Indeed, this appears to be the case. The blood-forming organs function well far into old age, whereas muscular weakness is one of the first signs of advancing years. It has been estimated that the human brain loses, without replacement, about 10,000 brain cells every day, so it is small wonder that mental ability decreases after some decades.

Interpretation of the Data

It is interesting now to return to the experimental findings to interpret them in the light of these ideas. First, it may be recalled that the experiments with nitrogen mustard led to the conclusion that the nondividing cells are responsible for senescence. Thus, it is necessary merely to alter the mutation theory to postulate that the mutations in the nondividing cells of the body are the cause of the aging phenomenon in animals.

Next, it is necessary to modify the simple mutation theory of aging to take account of the fact that after large doses of radiation young animals may have very high percentages of mutated cells but be far from senescent. Apparently the cells responsible for the aging of the animal can exist and function nearly normally for long periods, but not indefinitely, with badly damaged chromosomes. One can think of such cells as using the previously formed RNA of the cytoplasm to determine protein synthesis and cell function. Indeed, one could go a step further and postulate that there might be several times as much RNA present in a cell at the time of the mutation as is needed for minimal function. If the cell underwent cell division, each daughter cell would contain half the original concentration of RNA corresponding to the damaged DNA, and its full concentration of all other RNA. This process might be repeated several times before the concentration of this "deficient" RNA fell below the level necessary for maintaining the life of the cell, at which time all the daughter cells would die.

It is possible to produce an impressive amount of evidence to support these ideas. First, it seems clear that cells can indeed divide many times after the induction of a lethal mutation. Demerec (*22*) found that when a suspension of bacteria is irradiated, many of the cells continue to divide for as many as 12 generations before all the induced mutations become manifest. Newcombe and Scott (*23*) analyzed this phenomenon (phenotypic delay) and concluded that the mutations were indeed induced in the cells but that the influence of the damaged gene could remain dormant for many cell generations.

Furthermore, Puck *et al.* (*24*) showed that mammalian cells in tissue culture continue to divide, from three to five times, after a moderate dose of radiation before the daughter cells die or become bizarre. Cells from all mammalian organs behave in this way. Mammalian red blood cells function normally for a long time without a nucleus. It is interesting to recall, in this connection, the old experiments of Ethel Harvey in which sea-urchin eggs were centrifuged into two halves, a nucleate and a nonnucleate half. The "cell" lacking a nucleus continued to undergo cell division many times before it died. This is not to say it is necessary for a cell to undergo cell division several times before it dies a genetic death, but merely to point out the possibility of a considerably delayed genetic death.

There is also evidence that does not support the idea of a cell's functioning for a long time by means of RNA without DNA. Wulff, Quastler, and Sherman (*25*), using labeled RNA precursors, found quite a high incorporation

into nerve cells of the brain. This seems to indicate a rather rapid RNA turnover in these cells—a finding which implies that the DNA is actively synthesizing RNA and thus is essential for the day-to-day functioning of brain cells.

Wulff *et al.* (*25*) postulated that the basic problem in aging is the formation of faulty RNA which causes the synthesis of defective enzymes. This model certainly would explain the continued rapid turnover of RNA, but it would not explain the delayed appearance of damage to the genetic structure. Perhaps the apparent rapid incorporation of precursors into RNA may be explained as exchange reactions on terminal groups, or perhaps the cytoplasmic RNA undergoes self-replication. Thus, the case in favor of the idea that cell function may be carried on for long periods by RNA alone may not be completely proved, but the fact does remain that cells can function for extended periods without direct participation of DNA.

The modification of the mutation theory of aging suggested by the experiments and by the supporting evidence seems well justified in broad outline, although future experiments will no doubt demonstrate the need for further changes.

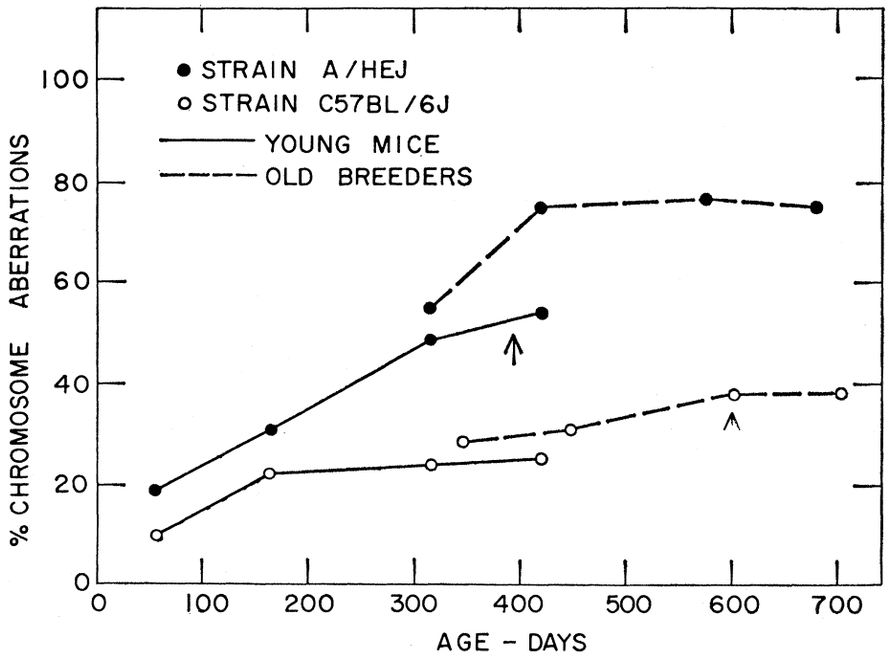

Fig. 8. Chromosome aberrations as a function of age in the livers of normal female mice of two strains having median life expectancies, as indicated by the arrows. Some of the mice were used for breeding until they were a year old, when they were first used in the experiment. The data show that short-lived mice develop spontaneous chromosome damage very rapidly. [Crowley and Curtis (*20*)]

Some Correlations

The results of the experiments indicate that somatic cells of the mammal develop chromosome aberrations at fantastically high rates. It is not possible to compute mutation rates from these figures, but some strains of mice exhibit grossly abnormal mitoses in 70 percent of all liver cells (and presumably an equal proportion in all nondividing cells) when the mice are hardly past middle age. Surely this must mean that virtually all cells carry many gene mutations. The wonder is that the mice are alive at all! If mutations occurred in the germ cells with anything like this frequency, the species could hardly survive a generation. Either the gametic cells are somehow protected against mutations or the process of meiosis preparatory to fertilization may weed out practically all defective cells.

Actually, such a situation is quite well known in plants, even though it cannot be explained at present. Many flowers have variegated petals consisting of spots of one color on a background of another color. Each spot represents a somatic mutation, so the spontaneous somatic mutation rate must be very high. Yet the species breeds true.

It is usually assumed, and the assumption seems eminently reasonable, that spontaneous mutations are caused by errors of replication. However, the nondividing mammalian cells are not replicating, and yet they are developing mutations at a rapid rate. We also know from the experiments with long-term, low-level x-irradiation that the chromosomes of resting cells can heal themselves, so one wonders if the spontaneous chromosome damage may not be very much higher than is indicated by the work discussed here, because many of the breaks may have healed before the time of observation.

It has been known for many years that, in both mice and men, the offspring of relatively old mothers have more defects and a shorter life span than the offspring of young mothers (see *26*). If the age of the father has an effect in this regard, it is very small. This has been very puzzling, but one can now postulate that since the ovocytes in the female stay in the ovary for a long time without undergoing cell division, they can accumulate mutations as time goes on, some of which can survive meiosis to endow the offspring with mutations. In the males the spermatogonia undergo cell division continually and thus do not build up mutations. Whereas this concept is certainly not proved, it fits well with the present theory.

A number of recent experiments have shown differences in time and mode of death associated with natural and radiation-induced aging (see *27*), and this has led some workers to conclude that a different biological process is operative in the two cases. The available evidence indicates that different organs contribute differently to the aging process, and that chromosomal damage takes a very long time to manifest itself in some organs. Furthermore, radiation is a well-known therapeutic agent for some diseases. Thus it would be surprising if radiation or any other agent which contributes to aging did so by a simple process of accelerating all the signs and symptoms of aging to an equal extent.

Some of these thoughts are certainly far from proved facts, and they are added merely to show some of the current thinking in this field, to indicate some of the consequences of the mutation theory of aging, and to suggest some of the areas for future research.

Summary

In this article the several theories put forward to explain the biological mechanisms underlying the aging process are examined. The only ones which attack the problem from the point of view of basic biological mechanisms are the wear-and-tear theory and the somatic mutation theory. The finding that radiation accelerates the aging process is a potent tool for attacking the problem experimentally. Experiments with mice specifically designed to verify the wear-and-tear theory showed conclusively that stress per se does not contribute to aging, and no experimental evidence could be found to support the theory.

On the other hand, a great deal of evidence now available indicates that mutations in somatic cells play a dominant role in aging. It is further shown that the organs having cells which frequently undergo cell division take part in the aging process very little, if at all. Organs having cells which seldom, if ever, divide have no opportunity to throw off either spontaneous or induced mutations, and it is these organs which are responsible for the aging of the animal. Spontaneous mutations build up at a rapid rate in these organs. A cell may continue to function normally long after it has suffered a deleterious mutation, and this accounts for much of the delay in the expression of radiation damage. It is suggested that the mutation rates for somatic cells are very much higher than the rates for gametic cells, and that this circumstance insures the death of the individual and the survival of the species (*28*).

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CURRENT PROBLEMS IN RESEARCH

The Structure of RNA

Reovirus RNA and transfer RNA have similar three-dimensional structures, which differ from DNA.

Robert Langridge and Peter J. Gomatos

The structure of deoxyribonucleic acid (DNA) was fairly well established in 1953, based on x-ray diffraction patterns, chemical analysis, and molecular model building (*1*). The very high quality of the diffraction patterns obtainable from DNA has enabled the structure to be refined in considerable detail (*2*, *3*).

The structure of ribonucleic acid (RNA) has proved to be a much more intractable problem. An extensive x-ray diffraction study (see *4*) showed that the RNA from a variety of sources gave diffraction patterns which were effectively identical, but were poorly defined and diffuse. Analytical and various physicochemical data did not provide much assistance in molecular model building, and the diffraction patterns remained uninterpretable for several years.

Robert Langridge is research associate at the Children's Cancer Research Foundation, and at Harvard Medical School, Boston, Massachusetts. Peter J. Gomatos is guest investigator at the Rockefeller Institute, New York.

Transfer RNA

The work of Spencer *et al.* (*5*) on a preparation made from amino-acid transfer RNA extracted from yeast, finally showed that the sodium salt of this RNA has a structure which is somewhat similar to the *A* form of the sodium salt of DNA (*2*) and that the diffuse patterns given by other RNA preparations could be accounted for by a disordered form of the type of pattern obtained from transfer RNA.

There is some question about the relationship between the structure observed and the native, transfer RNA molecule, since it has recently been shown that the method of preparation yields fragments having a molecular weight of slightly less than half that of the native material (*6*). There is, however, no question that the structure observed is the structure of double-helical RNA, and the observations to be reported here show that a preparation of RNA of very high molecular weight from reovirus gives diffraction patterns which are very similar indeed to those obtained from double-helical