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The Biology of Aging

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MASTER

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FOREWORD

The Brookhaven Lectures, held by and for the Brookhaven staff, are meant to provide an intellectual meeting ground for all scientists of the Laboratory. In this role they serve a double purpose: they are to acquaint the listeners with new developments and ideas not only in their own field, but also in other important fields of science, and to give them a heightened awareness of the aims and potentialities of Brookhaven National Laboratory.

Before describing some recent research or the novel design and possible uses of a machine or apparatus, the lecturers attempt to familiarize the audience with the background of the topic to be treated and to define unfamiliar terms as far as possible.

Of course we are fully conscious of the numerous hurdles and pitfalls which necessarily beset such a venture. In particular, the difference in outlook and method between physical and biological sciences presents formidable difficulties. However, if we wish to be aware of progress in other fields of science, we have to consider each obstacle as a challenge which can be met.

The lectures are found to yield some incidental rewards which heighten their spell: In order to organize his talk the lecturer has to look at his work with a new, wider perspective, which provides a satisfying contrast to the often very specialized point of view from which he usually approaches his theoretical or experimental research. Conversely, during the discussion period after his talk, he may derive valuable stimulation from searching questions or technical advice received from listeners with different scientific backgrounds. The audience, on the other hand, has an opportunity to see a colleague who may have long been a friend or acquaintance in a new and interesting light.

The lectures are being organized by a committee which consists of representatives of all departments of the Laboratory. A list of the lectures that have been given and of those which are now scheduled appears on the back of this report.

Gertrude Scharff-Goldhaber

The drawing on the cover is taken from a 5th Century B.C. relief on the Acropolis in Athens, the "Dreaming Athena," by an unknown sculptor.

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INTRODUCTION

I feel particularly honored, as a member of the Medical Department, to introduce someone from the Biology Department.

Our speaker tonight hails from the Middle West, the state of Michigan, and from the University of Michigan, where he received his undergraduate schooling; he then migrated East and has remained here ever since. After taking his M.A. at Swarthmore, he moved up to New Haven for his Ph.D. at Yale. Between the time of his graduation from Yale and 1950, he was at various times and for varying periods at Cold Spring Harbor, Columbia, Johns Hopkins, Oak Ridge, and Vanderbilt. At Vanderbilt, he was Professor and Chairman of the Department of Physiology in the Medical School from 1947 to 1950.

In 1950, primarily through the efforts of our own revered Donald Van Slyke, ably assisted by Leland Haworth, Dr. Curtis was offered the post of Chairman in the Biology Department here – an offer which, in the opinion of myself and many others, it was Brookhaven's good fortune that he accepted. The esteem in which the Biology Department is held both in the United States and abroad is clearly not due to a single individual; but the capacity for hard, conscientious work, the independence of mind, the obvious integrity of the Chairman, and his ability to pick good men and give them good support have played a major role in the growth and development of the Department.

The respect in which he is held outside Brookhaven is attested, for example, by the frequency with which the National Research Council and the National Institutes of Health have asked, and still ask, him to be on committees; he has been in the past, and continues to be, on the editorial boards of scientific journals and books, and is a member of many scientific societies, including The American Physical Society.

It may come as something of a surprise to many in the audience that Dr. Curtis took his Ph.D. at Yale not in physiology or some other branch of the life sciences, but in physics. And some of his early papers appeared in *The Physical Review* and *The Journal of Chemical Physics*. However, early in his career, the budding field of biophysics attracted him. During the period 1936 to 1942, Dr. Curtis with Dr. Kenneth Cole published a series of papers that had a seminal influence on modern research in the basic phenomena associated with cellular excitation – excitation probably being one of the criteria of all living cells. By a series of elegant experiments with microelectrodes, using an isolated nerve fiber from the squid, Drs. Curtis and Cole demonstrated that, when a nerve is excited, there is depolarization of the membrane at the point of excitation with reversal of the charge so that the outside becomes negative and the inside positive, with a similar phenomenon occurring down the nerve fiber as the impulse is propagated. On this work, that of Hodgkin and Huxley was built, for which they shared a Nobel Prize in 1963.

During the last eight years or more, Dr. Curtis has been engaged in the study of a phenomenon with which most members of this audience are all too familiar: the enigmatic process of aging. It is on this fundamental problem of all living matter that he will speak to us tonight.

LEWIS K. DAHL

The Biology of Aging

Death and taxes are things which people from the beginning of civilization have come to accept as inevitable, but, whereas we think we understand the necessity for taxes, why it is necessary to grow old and die remains largely a mystery. We all know persons who are relatively "young" at age 75 and others who are quite "old" at 55. The problem is clearly not one that has solely to do with the passage of time, but certainly time is an important factor.

I first became interested in this problem about 20 years ago, during the war, when there was much concern about the biological effects of the various radiations generated as a consequence of the development of atomic energy. All the ionizing radiations were found to have very profound and long-lasting effects on animals. If a mouse, for example, were given a large but nonfatal dose of radiation as a young adult, it might show no external sign of this insult, and careful physiological examination was needed to demonstrate that anything was wrong with the mouse. Even this measurable damage disappeared in a few days and the animals appeared entirely healthy. However, when we kept track of them we found that they died, on the average, sooner than the normal

animals. On examination we found that the causes of death were just about the same as for the controls, but the various diseases were contracted sooner. Furthermore the mice looked and acted old. This phenomenon is illustrated in Figure 1.

It thus appeared that radiation was speeding up the aging process. In attempting to explain why radiation acted in this way, we necessarily had to ask, what causes aging in the first place? The answers to this question were vague in the extreme. Thus we were led into the much broader question of aging and senescence in attempting to explain the radiation effects. However, in studying this broader question we were able to use radiation as a potent tool, since it was the only agent known that would mimic the aging process.

In studying aging one must first define the phenomenon. This may seem trite, but in truth gerontologists are still not completely agreed even on the definition of aging. It is certainly not just something that leads to death, because a wide variety of things cause death which have nothing to do with aging. Every exact definition requires an assumption relative to the mechanism of aging, and there is certainly no agreement on this.

Figure 1. Photographs of two groups of 14-month-old mice which originally had been identical. The control mice on the left are normal and healthy. The group on the right received a large but nonfatal dose of radiation when they were young adults; only three are left in this group and they are old and senile.

At the present time the question must be approached from a statistical point of view. Figure 2 shows survival curves for different populations, and three facts are quite clear from these curves. First, it is especially clear from the curve for U.S. populations in 1940 that death is not a chance phenomenon, otherwise the curve would have been exponential. As the individual grows older the probability of death increases. Second, as is well known, the average life span has increased markedly in recent years because of better sanitation, more food, better medicine, etc. Lastly, the curves show that the *maximum* life span is about the same for different races of man and has not changed appreciably in recent years – indeed it has probably not changed for many centuries.

If the death rate (the probability of dying at any age) is plotted as a function of age (Figure 3), it is seen to increase very rapidly after adulthood is reached. It appears to be an exponential function, and a plot of the log of the age-specific death rate against age (Figure 4) is almost a straight line. This is known as the Gompertz plot and shows that the probability of dying in any year is indeed very close to an exponential function of the age of the individual. The data shown are for all causes of death; but curves for specific causes of death also follow quite closely an exponential function with nearly the same slope on a Gompertz plot, although of course with vastly different absolute values. Thus the slope of the curve for death due to heart disease is not much different from that for death from leukemia or even tuberculosis.

The slope of the line in Figure 4 gives a doubling time of 8.5 years, and, since this is a straight line, it is independent of age beyond adulthood. In other

Figure 2. Percentage of survivors of male live births (modified from Comfort¹).

words, the probability of dying in any year doubles every time 8.5 years is added to the age. This probability of dying has been called the “force of mortality,” and this force keeps steadily increasing as we grow older.

This leads to the concept of physiological age as contrasted with chronological age. For any population the age-specific death rate at any age is a definite quantity and has often been taken as a measure of the physiological age of that group. This varies considerably from one group of persons to the next and, as we have seen, for all populations tends to increase markedly with chronological age.

This, then, can be taken as a definition of aging: It is that deteriorative process which leads to a continually higher probability that the individual will die. This is not a very satisfactory definition, but it is the best there is now.

It is interesting that the great advances in medical science have added many years to the life expectancy of younger people but have done very little for older people. This may be illustrated by plotting the age-specific death rate at two different times for the same stable population. Figure 5 shows that for young adults the death rate has recently been reduced by more than a factor of 3, whereas for very old people the probability of death has not changed appreciably in this century, and as far as we know it has never changed. In

Figure 3. Age-specific death rate from all causes as a function of age for U.S. males, 1949 (data from Jones²).

other words, modern man has a much better chance of living to a ripe old age, but if he does he will probably be about as senile as was a man of the same age a century ago. This indicates again that there is a ceiling on the life span, and, if the maximum span is to be increased, a completely different approach will be necessary.

THEORIES OF AGING

The problem, then, is what causes the deteriorative process. Many theories have been advanced but there has been a dearth of good experimental evidence with which to test them. Of the theories, the two that seemed to show the most promise were the wear and tear theory and the somatic mutation theory.

The first of these theories postulates that the organism simply "wears out" like an automobile, or in other words has only a limited capacity for self-repair. A variation on this is Pearl's "rate of living" hypothesis,⁴ which postulates a given amount of vital substance for each individual, and when that is used up life comes to an end. Selye³ and Jones² have put forward the stress theory rather forcefully.

To consider first the rate of living idea, Rubner⁵ showed many years ago that all mammals have about the same metabolism per gram of tissue per lifetime. In other words, a gram of tissue in a mouse has about the same energy expenditure in 18 months as does a gram of human tissue in 70 years. In more modern terminology, one could postulate that when mammalian cells differentiate to form the total organism they are endowed with a certain complement of enzymes, and these are used up in proportion to the metabolism of the cell. Pearl and many others have observed that the life span of adult *Drosophila* can be regulated over wide ranges depending on the external temperature which controls their metabolism. More recently Carlson⁶ and Johnson¹⁸ have kept rats at cold temperatures, which requires them to increase their metabolism considerably to maintain the body temperature. The life span of these rats was considerably reduced, and they died of the same diseases as did normal rats. On the other hand, McCay²¹ found that rats kept on restricted diets, so that they could not grow nearly as large as the controls, lived much longer than the controls. The metabolism per gram was somewhat higher in the

Figure 4. Log age-specific death rate as a function of age (same data as in Figure 3).

Figure 5. Log age-specific death rate vs. age for males in Sweden for 1900-1910 and for 1951 (data from Jones²).

restricted diet rats, and this would argue against the metabolism concept. These are very important observations, and ones to which I would like to return later.

The stress concept has a much less definite experimental basis. Selye has observed conditions in his stressed rats which appear spontaneously in older rats. However, since he has never conducted longevity studies on stressed animals, his observations relative to aging are largely conjecture.

Jones has made an extensive analysis of vital statistics from various populations of the world. He finds, as is well known, that the average life span is increasing dramatically all over the world as modern sanitation, better diets, medical care, etc., are being introduced. However, there are still wide differences in this respect in various parts of the world. From a study of the statistics he concludes that in any population, as it becomes healthier, all diseases are reduced about equally, not only the communicable diseases. He reasons that the general vitality of the population is improving to give a decreased mortality for all diseases. Populations with a high incidence of disease in the younger age groups have a decreased resistance to disease in the older groups. It is as if one disease predisposes an individual to another disease later. This leads directly to the idea that senescence is caused by the summation of the insults which the individual receives.

However, I feel one should not take this as proven by any means. By choosing other popula-

tions to study one can come to contrary conclusions. Also, populations do not "stand still" like a colony of mice living under controlled conditions. Thus Jones' studies suggest his conclusions but do not prove them.

This theory is one which would seem relatively simple to explore from an experimental point of view. We undertook several years ago to find out whether or not stress which did not kill an animal, and from which it could apparently completely recover, would shorten the life span. As previously indicated, ionizing radiation seems to do just that, so an easy way to explain radiation-induced aging is merely to say that it acts as a severe stress. To test this, however, we tried other stresses.

It was necessary to use a stress that could be quantitated; therefore, we used chemicals that could be injected in exact amounts. We were also careful to use stresses that would be as nonspecific as possible, so that all organs of the body would be subjected to them.

Figure 6 shows the results of one such experiment, in which large doses of the agents were given to groups of mice, and the longevity of these mice was compared with that of a group of mice receiving a large dose of radiation. No decrement in life span can be seen in the chemically treated mice as compared to the normal controls, but a dramatic decrease in life expectancy results from an equally severe radiation stress. This quite conclusively proves that radiation does not shorten the life span because of its stress-producing qualities, but must do so for some other reason. But this experiment does not prove that a stress may not contribute to senescence if it is severe enough and repeated often enough.

Figure 6. Survival curves for groups of mice given the stress agents indicated. No decrease in life span is seen from large doses of typhoid toxoid or nitrogen mustard, but there is a dramatic decrease due to ionizing radiation (from Curtis²⁰).

Figure 7. Survival curves for mice that received massive but nonlethal doses of the chemicals indicated every 14 days for a large part of their lives. Even such drastic treatment did not decrease their life expectancy (from Curtis and Gebhard⁷³).

We therefore initiated a series of experiments designed to apply chemical stresses repeatedly over a large fraction of the animals' life span. The data in Figure 7 are from an experiment representative of this series. A large but just nonfatal dose of the agent was administered each week (or other suitable interval). Because the experimental animals built up immunity to these agents, another identical group had to be tested each week to determine the barely sublethal dose. This amount was then given to the experimental group, and, as seen from the curves, there was no effect on the life span. Other agents used similarly included typhoid toxin, typhoid toxoid, nitrogen mustard, and subcutaneous and intraperitoneal injections of turpentine. All of these were repeated as often as we dared, and all had no effect on the life span.

These experiments all showed rather conclusively that a stress, *per se*, is not an agent contributing to the basic aging process. This finding completely fails to substantiate the belief held by many people for many centuries that, if a person contracts a serious disease and recovers from it, the experience has nonetheless shortened his life expectancy. And it gives no experimental foundation to the very plausible ideas of Jones, Selye, and others.

Figure 8. 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 chromosome which do not take part in the mitosis. In a normal mitosis all chromosomes pull apart cleanly (from Curtis¹³).

THE SOMATIC MUTATION THEORY

Turning now to the somatic mutation theory, one finds quite a different outlook. The idea behind the theory is quite old but only recently has it been formulated in definite terms. Failla,⁸ Curtis and Gebhard,⁹ and Szilard¹⁰ have pointed out that the somatic (body) cells of an animal should be just as subject to mutation as germ cells, and, since practically all mutations are deleterious, the body cells would become increasingly inefficient as they developed these mutations. The organs of which they were a part would likewise become inefficient and therefore increasingly unable to withstand disease.

Radiation is about the most potent mutagenic agent known, and the fact that it seems to speed up the aging process is a very forceful argument in favor of this theory. Both Szilard and Failla have offered theoretical treatments favoring the idea, but no experimental data to support it.

Several years ago we undertook a program designed to produce experimental evidence bearing on this theory. The main obstacle had been the lack of a valid method for directly measuring mutations in somatic cells. The difficulty, of course, is that a somatic cell cannot form a second generation, so that a mutation, if it is produced, can never be brought to light. We tackled the problem indirectly. In plants it is well known that the germ cells differentiate from the somatic cells; therefore, mutations produced in the somatic cells of a plant can be scored by growing the plant to maturity to

Figure 9. Chromosome aberrations in liver cells as a function of age in normal mice and in mice which had received a large dose of x rays (from Stevenson and Curtis¹⁴).

produce seed, growing the seed, and scoring the mutations in the usual way. Also, the somatic cells of the original plant can be examined for abnormalities in the chromosome structure which might give a clue to the presence of a mutation. Caldecott¹¹ has done this for barley plants and finds a straight line relationship between chromosome aberrations produced by radiation in the somatic cells and true mutations as scored in the subsequent generations. This should also be true of animals, so that if a score of chromosome aberrations in the somatic cells of an animal could be obtained it should give an index of the mutations present.

A scoring method was developed for liver cells following work by Albert¹² and others. The cells of the liver practically never undergo division, but they can be made to do so if part of the liver is destroyed, in which case they will divide rapidly until the liver is restored to normal size. The technique is to give a mouse a dose of carbon tetrachloride designed to destroy about 60% of the liver, and 72 hours later, at the height of the regeneration, to sacrifice the mouse and prepare a bit of the liver for microscopic examination. Only those

Figure 10. Chromosome aberrations in liver cells of mice (1) subjected continuously to gamma irradiation, (2) given a large single dose of x rays, and (3) kept as controls. The dashed line shows the rate of build-up of chromosome aberrations which would be expected on theoretical grounds if low dose rate irradiation were as effective as a single acute dose. The experimental curve is far from this theoretical curve, and indeed is such as to indicate that the low dose rate irradiation is only about 25% as effective. From this it is concluded that at least under some circumstances it is possible for mammalian chromosomes to heal themselves (from Curtis and Crowley¹³).

cells are scored in which the nuclei are just pulling apart in the late stages of cell division (anaphase and early telophase). At this critical point in cell division, if the chromosomes are not in perfect condition, some abnormality will be observed. Usually either the chromosomes do not pull apart cleanly and thus form bridges between the daughter cells which eventually break, or else pieces of chromosome are broken off which do not take part in the division at all. One such abnormal cell is shown in Figure 8.

By using this method it has been possible to obtain a good deal of evidence bearing on the mutation theory. First, a steady increase was noted in chromosome aberrations with age.¹⁴ This is shown in Figure 9 and is a consistent finding in all experiments. When the experimental mice were 12 months of age, the aberrations reached 22%. If 22% of all cells were grossly abnormal on microscopic examination, it seems safe to assume that virtually all cells carried at least one mutation at that time.

Next it was found that following a single dose of x rays large enough to kill half the animals acutely, the remaining animals exhibited very large numbers of aberrations, which declined slowly over a period of months (Figure 9).

It has been known for many years that the administration of x or gamma rays at a very low dose rate is only about one quarter as effective in

Figure 11. Chromosome aberrations in liver cells of mice (1) given a large single dose of neutrons, (2) subjected to neutron irradiation at a low dose rate, and (3) kept as controls. If these two dose rates of neutrons are equally effective in causing chromosome aberrations, the number of aberrations will be the same (the curves will cross) at 43 days, and it can be seen that such is the case. From this it is concluded that a chromosome is not able to heal itself from the severe damage caused by a hit from a neutron (from Curtis, Tilley, and Crowley¹⁶).

shortening the life span as is the same dose administered acutely. A series of experiments¹⁵ showed that gamma rays administered at a low dose rate cause chromosome aberrations to develop quite slowly, and indeed for equal doses are only about a quarter as effective in producing chromosome aberrations as are single doses (Figure 10).

On the other hand it is well known that neutrons administered at low dose rates are equally effective in shortening the life span as are the same numbers administered acutely. Results of a recent experiment¹⁶ indicated that the same is true for the development of chromosome aberrations (Figure 11).

It should also be noted from these and other curves that for equal doses neutrons are about twice as effective in producing chromosome aberrations as are x rays. This is also the ratio of effectiveness of these two radiations in producing shortening of the life span.

Thus, for all types of radiations and radiation regimes the degree of life shortening produced is quantitatively related to the amount of chromosomal damage produced by each. It would be a rare coincidence if there were not a causal relationship between them.

It is well known that two different inbred strains of mice may have quite different life expectancies even though both may die of roughly the same diseases. In a recent experiment with two such strains it was shown¹⁷ that the long-lived strain

Figure 12. Chromosome aberrations in liver cells of two inbred strains of mice, plotted as a function of age. The median life span of each strain is indicated by the arrows. In each case, the solid lines represent data on animals 8 weeks old at the start of the experiment and the broken lines, on old breeders about 1 year old at the start of the experiment (from Crowley and Curtis¹⁷).

(C57BL/6J) developed chromosome aberrations at a much slower rate than the short-lived strain (A/HEJ) (Figure 12). This difference is very striking and strongly supports the belief that chromosome aberrations are the cause of aging.

Since radiation obviously is not the only mutagenic agent, administration of chemical mutagens might also be expected to shorten the life span. To test this idea, a series of experiments was undertaken with the mutagen nitrogen mustard. That this drug breaks chromosomes in bone marrow and other organs is known and is the basis for its use in the therapy of leukemia. A single administration of nitrogen mustard had no effect on the life span of mice. Consequently, a group of mice were given an almost lethal dose twice a week throughout their life span; their life expectancy was unchanged by this very drastic procedure. The chromosome aberrations were also measured in the livers of these mice¹⁴ and virtually no increase in aberration rate was found (Figure 13). Thus one is led to conclude that this agent either does not reach or does not affect the cells of the body like liver cells which normally do not undergo cell division.

EVALUATION OF THE THEORY

All the evidence so far points to a relation between chromosome aberrations and aging, and

Figure 13. Chromosome aberrations in liver cells of mice given nitrogen mustard at two different levels twice a week for the duration of the experiment, and of controls (given saline). Data from a group of mice given a large single dose of x rays are also shown for comparison. This drastic nitrogen mustard treatment increases the aberration rate only slightly, whereas a single dose of radiation increases it very markedly (from Stevenson and Curtis¹⁴).

Figure 14. Chromosome aberrations in liver cells of both irradiated and control mice, and with and without carbon tetrachloride injections every 45 days (arrows) which destroyed about half the liver. The induced mitosis caused only a very slow return to control levels. The standard error for each point is indicated (from Curtis, Tilley, and Crowley¹⁹).

furnishes strong support for the somatic mutation theory of aging. It thus appears that the deterioration which takes place with aging is actually within the chromosome structure of the cell. However, the simple theory postulates that when a certain percentage of the cells of the body develop serious mutations, senescence and death follow. The above experiments clearly indicate that this theory is correct in its basic concept, but the simple theory must be changed in at least two important ways.

First, the nitrogen mustard experiments demonstrate that an agent which seriously affects the dividing cells of the body such as bone marrow, but does not affect the nondividing cells such as liver, does not produce senescence. It seems reasonable to conclude that this agent killed off a large fraction of the bone marrow cells, but the few remaining healthy stem cells underwent division very rapidly and replaced the killed cells. The bone marrow cells carrying mutations will probably be eliminated by cell selection during cell division because of the competition for survival.

On the other hand the nondividing cells of the body do not have this opportunity. A mutation will persist in these cells, causing them to function

badly. On this basis it is the organs containing such cells which are responsible for senescence. The organs, like bone marrow, which have continually dividing cells could be considered immortal in the same sense as is a well nourished bacterial culture. However, the organs with dividing cells are subject to cancer induction, so that one evil is merely traded for another. If it is postulated that cancer is caused by a mutation, which seems eminently reasonable, then it becomes clear that mutations are responsible for all the deteriorative processes which take place with age, and this explains why all forms of disease seem to be accelerated by the deteriorative process we call aging.

However, it is apparent that things are not quite so simple. When an animal receives a dose of x radiation, the aberrations rise abruptly but then fall over a period of some months, and it is important to understand the reasons for the decline. If it were to be explained on the basis of cell selection during cell division, quite high mitotic rates would have to be postulated for liver cells.

An experiment was undertaken to investigate this point. A group of mice was given a dose of radiation; then both the experimental animals and

the controls were divided, and one group of each was given carbon tetrachloride injections every 45 days which destroyed about half the liver and caused regeneration.¹⁹ If cell division is very effective in eliminating aberrations, the carbon tetrachloride treated animals should have eliminated them very rapidly. The results (Figure 14) show that, whereas aberrations were eliminated more rapidly than normal, they were not eliminated nearly fast enough to explain the decline following x irradiation. The conclusion seems inevitable that chromosomes can heal themselves over a long period of time. The chronic irradiation studies showed that more than 75% of the aberrations formed by a low dose of radiation are healed very quickly. On the other hand, the neutron studies showed no apparent healing following this type of radiation. The conclusion from all this is that, if the cell is not required to undergo division, small chromosome injuries can be healed in a matter of minutes, more severe ones in weeks or months, and very severe ones are never healed.

These experiments leave us with a picture of the chromosome as a very labile structure which is apparently continually breaking down and repairing itself. Since aging is dependent on chromosome stability, the repair process is as important as the factors responsible for the injuries. This picture also gives us great hope for the future, because our increasing knowledge of protein structure and DNA structure should soon make it possible to devise methods for stabilizing chromosome structure. It looks as though the difference between people who live to be very old and those who die young may be in the relative stability of the molecular structure of the chromosomes.

Another very interesting conclusion from these experiments is that there is apparently a very long delay between the production of a mutation and its expression. Thus, when very young mice are given a dose of radiation, the chromosomes of most of the body cells are badly disrupted, but the animals appear young and are in very good health. They must live half or more of their life spans before the consequences of this damage become apparent. This is difficult to explain, but in the light of modern concepts of the role of DNA in cell function it may be possible to do so.

Figure 15 shows a schematic representation of a cell, showing that each specific DNA molecule is unique and responsible for the synthesis of a particular protein. There are thought to be one DNA

Figure 15. Schematic representation of cellular function. The different proteins of the cell are synthesized from specific DNA molecules as indicated. The destruction of a single DNA molecule will eventually lead to the elimination of a class of proteins from the cell.

molecule, 10 to 100 RNA molecules, and more than 1000 protein molecules of each species. In the simplest concept of a mutation, a single DNA molecule is destroyed, which means that eventually there will be none of the corresponding protein. If this protein is vital to the life of the cell, the cell will die as soon as all the corresponding RNA and protein are exhausted. This would be a lethal mutation. A nonlethal mutation would merely lead to a changed protein complement, which might merely mean a changed metabolism. It may take some time for the RNA and protein to become exhausted after a particular DNA is destroyed, and there is at least some evidence that this may be a very long time indeed. There is good evidence indicating that a cell may even undergo cell division several times after receiving a lethal mutation before the cell line goes to pieces.²⁰ It thus appears that the nucleus of the nondividing cell of the mammal exerts only very occasional control over the function of the cell after it is formed, but it does eventually make its presence, or absence, felt.

It appears then that, whereas much remains to be done, the general features of the somatic mutation theory, as modified, seem reasonably well substantiated. In addition, it now seems clear why it is that radiation produces effects in the mammal which are so long delayed.

CORRELATIONS

This theory explains a number of well-known phenomena very nicely. For example, it has been known for many years that the offspring of old mothers have more defects and live a shorter life span than those of young mothers; mongolism, which is known to be caused by a genetic defect, is many times more prevalent in children of mothers

over age 40 than in those of younger ones. If the age of the father has any effect in this regard it is quite small. On the basis of the present evidence it can be postulated that, since the oocytes in the female stay in the ovary for years without undergoing division, they have no opportunity to throw off mutations. They can accumulate mutations as time goes on, some of which survive meiosis to endow the offspring with mutations. In males, spermatogenesis is in progress continually, thus mutations tend to be eliminated by cell selection.

It was indicated above that, although the rate of living theory does not have any real proof to substantiate it, enough pieces of evidence suggest its validity that one hesitates to discard it. It can now be seen that this concept fits well with the somatic mutation theory. There must be a long time between the production of a mutation and its manifestation in terms of cell function, and during this time the cell is functioning by means of its stored RNA and proteins. If the proteins are being used up faster by an increased metabolism, the mutation will become evident sooner. On this basis there would seem to be no conflict whatever between these two theories.

The results of these experiments do not allow a computation of the mutation rates in somatic cells, but even in the normal old animal the true rate must be fantastically high. If the rate in gametic cells were anything like as high, even within several orders of magnitude, the species could not survive more than a few generations. The gametic cells must have very much more stable chromosomes than somatic cells, or else meiosis constitutes a very rigid screening process which allows only genetically perfect cells to get through.

There is no doubt that death is a tragedy for the individual, but a moment's reflection will convince one that it is necessary for the good of the species. The fact that the somatic mutation rate is so much higher than the gametic mutation rate may be nature's method of ensuring the death of the individual and the survival of the species.

SUMMARY

Ionizing radiation accelerates the aging process and has been used as a tool to study the biological mechanisms of aging. Aging is a slow deterioration with time which manifests itself by an increased susceptibility of the organism to all kinds of disease. Of the many theories of aging, the wear and

tear theory and the somatic mutation theory seemed most promising. A series of experiments has shown that stress *per se* is not a factor in aging. A method has been developed for measuring somatic mutations in mice, and it was shown that in all cases tested so far there is a quantitative correlation between aging and the development of somatic mutations. The simple somatic mutation theory of aging must be modified to account for the long delay between the production of a mutation and its effect on shortening life expectancy. This modified theory correlates many phenomena known to be associated with aging.

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