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MASTER**CESTI PRICES**H.C. \$ 1.00; MW 50**RADIATION AND AGING***

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RELEASED FOR ANNOUNCEMENT
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It has been known for many years that a dose of ionizing radiation administered to an animal will shorten its life expectancy by an amount proportional to the radiation dose (Storer, 1965). When one examines the causes of death it is found that, in general, irradiated animals die of the same diseases and in roughly the same proportion as normal animals, but they contract the diseases sooner (Lindop and Rotblat, 1961). There are some minor exceptions to this which can easily be explained. This has led to the assumption that radiation accelerates the aging process, and a controversy has arisen as to whether this is true, or whether the radiation is merely killing the animals by inducing the same diseases by an unrelated mechanism.

Implicit in this question are several assumptions concerning the nature of the aging process as well as the effects of radiation on animals and their reactions to it. Before one can intelligently discuss this question one must then define the terms more carefully, and understand the nature of the biological mechanisms involved.

The present paper will show that when our understanding of these mechanisms is applied to this problem, the question itself becomes rather trivial. It will then be shown that by the application of the results of the radiation experiments, a great deal has been learned concerning the biological mechanisms of aging. Based on this, one can then formulate a generalized, conceptual theory of aging.

THE NATURE OF AGING

Life proceeds according to a definite program which is unquestionably coded on the genes of the individual somatic cells. In this process each cell plays its assigned role and in general the processes of maturation, puberty, etc. proceed smoothly. But at the same time deterioration is taking place in the machinery of the body, and this deterioration seems to take place at an ever-increasing rate. It is sometimes difficult to distinguish between these two phenomena, and both of them or a combination of them have been referred to as aging. Some facets of development and maturity clearly belong in one category, some in another, and for some it is impossible at present to assign the category. For example, it is not known whether the lens of the eye loses its power of accommodation with age because of a genetically programmed aging or a cross-linking of the elastin fibers which may take place in spite of attempts on the part of the cells involved to prevent it.

Certainly we must class the degenerative diseases as examples of the deteriorative process which proceeds independently of the genetic program. Cancer, the autoimmune diseases, the cardiovascular diseases, etc. are examples clearly falling in this category and it is these diseases which are largely responsible for the eventual demise of the individual. The present paper will focus attention on these diseases, but this in no way implies that other aspects of the aging problem are not important.

Even narrowing the problem down this much still leaves a vast area to be covered. Certainly the events leading to cancer are quite different

from those leading to atherosclerosis. Even different kinds of cancer undoubtedly have quite different etiologies. Nevertheless these degenerative diseases seem to have many features in common, and it is this unifying aspect which will be considered here.

An additional note of caution should be sounded. Every plant and animal goes through its life cycle of development, maturity, and eventual death. There are certain features that all of these have in common, but also many features which are unique to the individual phylum, species and even variety. Even one strain of mice does not age in the same way as does another. But when one attempts to compare aging in a drosophila imago, for example, with human aging, the similarities become very obscure and conclusions drawn from work on one should be applied to the other with extreme caution. If such caution had always been exercised in the past, much of our present confusion might have been avoided.

A mammal is a complex biological mechanism, infinitely more complex than the most precise piece of machinery yet devised by man. There are many different reactions which are age dependent, and thousands of different ways in which the mechanism can malfunction. It is then a foregone conclusion that there is no single cause of aging, and the process must be analyzed in its entirety if one is to progress in the field.

Before considering the question of the acceleration of aging by radiation it will first be necessary to briefly review the known facts of the effects of radiation on mammals.

THE ACTION OF RADIATION ON CELLS AND TISSUES

If one irradiates a chemical substance, for example a protein either dry or in solution, it is found that it takes enormous doses of radiation to effect a measurable change. Yet a mammal may be killed or have his life appreciably shortened by relatively small doses. If one computes the maximum number of molecules which can be destroyed by such a lethal dose, one is led to the conclusion that perhaps only one in 10^5 molecules in a cell would be destroyed. Surely most cell systems could stand this degree of depletion without harm, except for the genetic system. The DNA molecules are probably present in the cell as one of a kind, so destruction of only one of them might mean the eventual loss of some vital function for the cell. This has led to the concept that most delayed radiation damage is due to injury to the genetic apparatus of the cell. Many pieces of evidence point strongly in this direction, a few of which will be briefly reviewed.

Chromosome aberrations can be scored in regenerating liver cells and this has been taken as an index of the mutations present in the somatic cells of the animal (Curtis, 1966). By means of this technique it has been shown that aberrations increase steadily with age in normal animals, reaching quite high percentages in older animals. Following a dose of x rays the aberration percentage increases dramatically in proportion to the size of the dose, and then returns to control levels over a period of many months in the mouse (Fig. 1).

Dose rate is also a very important variable. For x rays, if a given dose of radiation is delivered at a low dose rate, it will be much less effective in producing chromosome aberrations than if the same dose is delivered at a high dose rate. However, for low energy neutrons, the number of aberrations produced is independent of the dose rate. These experiments then demonstrate that the amount of genetic damage produced in the somatic cells of an animal depends in very important ways on things other than the total radiation dose delivered.

When one examines the amount of life shortening produced by each of these treatments it is found that it is not necessarily proportional to the dose of radiation, but in all cases is proportional to the amount of chromosomal damage which a given treatment produces in the animal. This, taken together with other similar evidence, gives very strong presumptive evidence indicating that the life shortening effect of radiation is due to its ability to cause genetic damage in the somatic cells.

Most studies of the causes of death in normal and sublethally irradiated animals have shown that the causes of death are about the same in both groups. However, the irradiated group contracts the diseases at a younger age (Lindop and Rotblat, 1961). All causes of death are not moved forward by the same amount, and indeed in exceptional cases the onset of disease may be delayed.

When other facets of the aging problem are examined it is found that the response to radiation is even more variable. For example, apparently the age-related changes in accommodation of the eye are quite unaffected

by radiation, and other reactions are probably only marginally affected by indirect processes.

The available evidence would then indicate that the delayed effects of radiation in the mammal are due entirely to the damage it may produce in the chromosomes of the somatic cells, but many mammalian cells can perform their assigned functions quite well with a large amount of genetic damage.

RADIATION AND AGING

At this point the question "does radiation accelerate aging" resolves itself into the question "which facets of the aging syndrome are affected by mutations in somatic cells and how much". The answer to this latter question seems quite clear: some facets are greatly affected, some very little, some not at all, and some of the symptoms of aging are even delayed. Radiation therapists have been capitalizing on this latter quality for many years. However, it does seem that most of the degenerative diseases which lead to the death of most men and experimental animals, are caused in part by mutations and are thus accelerated by radiation.

For these reasons this question does not seem worth pursuing further. However, these techniques have thrown considerable light on some facets of the aging problem, and these will now be examined.

MUTATIONS AND AGING

The mutation hypothesis of aging is quite old and has been extensively discussed in recent years (Curtis, 1966). There is very little

question but that (1) at least a few spontaneous mutations occur in mammalian somatic cells; (2) for practical purposes all such mutations are deleterious; and (3) they can be of some importance in the induction of some or all of the degenerative diseases. The question is then a quantitative one--how important are such mutations in natural aging.

Recent studies, using the technique of examining chromosome aberrations in liver cells, have shown a remarkable correlation between the rate at which chromosome aberrations (mutations) accumulate in animal cells, and the life span of the animal. Figure 2 shows an experiment in which the rate of accumulation of chromosome aberrations is compared for two inbred strains of mice; one long-lived and one short-lived. It will be seen that in a general way the short-lived mice accumulate mutations much faster than the long-lived ones. This relationship has since been confirmed for other inbred strains of mice, and this evidence, taken together with the results of the radiation experiments, leads one to believe that animals of short-lived strains die sooner because their chromosome structure is not very stable and the resulting mutations cause degenerative diseases to develop sooner.

Carrying this idea a step further, Fig. 3 shows similar results obtained on dogs, and on the same graph the results obtained on a number of strains of mice are indicated. It will be seen that dogs develop aberrations very much more slowly than mice and of course live much longer.

It is interesting to speculate about the reason why a liver cell in a dog should maintain a stable chromosome structure for many years whereas the mouse chromosomes break down in less than two years. However, nothing

is known at the present time about why this occurs, so it will have to be accepted as experimental fact. But again one is very tempted to feel that mice age rapidly because their chromosome structure is not very stable.

This experimental method has rather severe limitations, as illustrated by an experiment performed using mice of strain C58 (Curtis et al., 1966). These mice almost all die at about ten months of age of leukemia, and this genetic trait presumably arose as a single gene mutation in a normally long-lived strain, C57. A possible explanation of this disease assumes a heritable weakness at a particular point in the chromosome structure, and a break at that point leads to leukemia. This would explain why most, but not all, of the mice develop leukemia. However, the results of this experiment (Fig. 4) show that this simple explanation of penetrance is not tenable, at least in this instance, and the leukemia is probably caused by a point mutation. This indicates that the method has some quantitative limitations, and these should be kept in mind when interpreting data obtained by this method.

These experiments then give very strong presumptive evidence to indicate that the animals age, in part at least, because they develop mutations. One could take the view that if the chromosomes of a mouse were stabilized to the same extent as those of a dog, the mouse would live as long. This would suggest that mutation induction is the only factor in the determination of the average life span. This is unquestionably a gross oversimplification of a complex process. But it would be equally foolish to deny mutations a major role in the aging process.

The mutation hypothesis has been attacked on the ground that there is much too long a delay between mutation production and its manifestation in terms of life shortening. It is quite true, as seen in Fig. 1, that the mutations induced by a dose of x rays seem to gradually disappear, so by the time the effects of the radiation are apparent in terms of life shortening, the mutations have all but disappeared. It is then quite clear that a given level of mutations in the somatic cells does not indicate a certain physiological age. The aging process is much more complex than that. It is also well known that radiation delivered late in life is not nearly as effective in shortening life span as is the same dose delivered when the animals were young. Such considerations have led some to feel that mutations, at least as measured by chromosome aberrations, are probably of little importance in the reaction to radiation. However, this attitude ignores the large body of evidence linking chromosome aberrations and life shortening. One only has to assume a long delay, on the average, between mutation production and manifestation. There is a large body of evidence to support this assumption (Curtis, 1963), and, as will be shown later, such a delay correlates very well with other known phenomena of aging.

The two major problems then seem to be (1) to elucidate the relation between mutations and other factors in the aging process and (2) to explain the long delay between mutation induction and its manifestation.

A COMPOSITE CONCEPT OF AGING

The view adopted here, that aging is an increased probability of contracting one of the degenerative diseases, then demands a better understanding of this etiology. It is very poorly understood for all these diseases, but present indications are that they all proceed along somewhat parallel lines. The one which has received the most attention and is best understood is cancer, so this will be taken as a model.

Many experiments have indicated the multistep nature of this disease. Berenblum and Trainin (1963) have repeatedly emphasized this, and indicated that there must be at least two and probably many steps in the process. The experiments of Cole and Nowell (1964) support Berenblum's ideas and strongly suggest that perhaps the first two steps in the process would be the induction of a mutation followed by a strong stimulus for cell division. There must be other steps, but their nature is very poorly understood. For example, it is known that tumors arise mainly in tissue of a "precancerous" nature, and yet the evidence is accumulating that cancers arise from single cells (Linder and Gartler, 1965). Thus a change must have taken place to create a favorable environment for the development of a tumor, and another change (mutation?) in a cell in this environment which started the actual tumor. It is further well known that a tumor may remain benign for a very long time before a change suddenly takes place which converts it into a malignant tumor. It seems quite clear that all these changes are not mutations, but equally clear that some of them are.

The multiple factor concept of the etiology of the other degenerative diseases is less firmly established, but there is every reason to believe that the same basic principles apply. Certainly for the autoimmune diseases, it appears that a mutation is probably the first in a series of steps (Walford, Sjoarda and Anderson, 1965), and there is at least some evidence for this concept for the other degenerative diseases.

If one accepts the multistep concept, then many of the facts and theories of aging fall nicely into place. One is certainly not able to speak of "the" cause of aging. It must be clearly recognized that the mammal is a complex mechanism which may malfunction in a great many different ways and for a variety of reasons. Under this concept then one should speak only of the relative importance of the many factors which lead to senescence and death.

One of the most striking features of the degenerative diseases is the very rapid increase in incidence with the age of the individual. All the different diseases show roughly the same pattern of increase, although the absolute incidence may vary considerably from one disease to another (Kohn, 1963). This gives a very strong indication that the same sort of mechanism may be operative in the different diseases. It also shows that if the spontaneous mutation rate is constant, then the same sort of delay must be present for these mutations as was indicated for the radiation induced mutations. When the consequences of this composite concept are applied to the mortality curves, it is found that these relationships are very nicely explained.

AGE RELATIONSHIP OF DISEASE

Following up the above ideas, it is seen that the occurrence of a degenerative disease in an individual must be put on a probabilistic basis. Even for mice of strains which contract a fatal leukemia in 90% of the population at an early age, one is merely increasing the probability of leukemia. Since it is well known that mutations occur with a certain probability (mutation rate), the concept fits this theory very well. Each of the other influences also has a certain probability of occurrence. One can then represent the degenerative diseases by a scheme such as shown in Fig. 5. For each disease, a cell or group of cells must undergo a series of changes before the disease becomes manifest. The nature of all of the steps is not known but they are probably mostly chance occurrences which carry a certain probability. The rate constants α , β , γ , etc. are expressed as events per year, whether they be mutations or other type changes.

Other reactions not associated with probabilistic events certainly also occur as a function of age. Prominent among these is the cross-linking of collagen (Kohn, 1963), the feedback mechanisms within the cell which regulate RNA and protein synthesis (Curtis, 1963), and the physical change in DNA binding (von Hahn, 1966). These must all play roles but are probably secondary to the other factors as far as causes of death are concerned.

The multiple factor approach to the etiology of the degenerative diseases is by no means new, and has been ably reviewed recently by Burnet (1965). It was first expressed in quantitative terms by Armitage and Doll

(1954) who showed that a process such as depicted in Fig. 5 leads to the prediction that the death rate for any disease is a power function of the time, i.e.

$$\frac{dN}{dt} = Kt^{(n-1)} \quad (1)$$

where N is the percentage of persons contracting the disease, t the time, n the number of steps in the process and K a constant depending on the probability or mutation rate for each step, the population under study, the environment, etc. Each step carries a certain probability (mutation rate?) which is assumed low so the number of cells originally at risk is always large compared to the cells which actually make a crucial transition.

It is very remarkable how well this equation fits the known facts, and in Fig. 6 data for death rates for all causes for United States in 1963 is presented together with points derived from equation (1).

Turning to the individual degenerative diseases, Armitage and Doll (1954) showed that for most forms of cancer the equation fits the facts remarkably well. The only exceptions were those which are strongly dependent on the environment such as lung cancer or those strongly influenced by a changing hormonal concentration such as cancer of the breast. These exceptions are very important in showing that a mutation concept alone is not enough to account for the facts but one must also take environmental and other factors into consideration.

For the autoimmune diseases, Burch (1963a) has shown that the disease incidence fits a mutation concept remarkably well. In this instance

he finds it necessary to assume that the number of cells at risk is quite small so eventually a person will run out of mutable stem cells capable of forming a new "forbidden" clone so the incidence of the disease decreases late in life.

It has even been shown by Burch (1963b) that cardiovascular and other diseases seem to fall within the framework of the autoimmune diseases, and thus have somatic mutations as important parts of their etiology.

In order to explain the incidence of these diseases by this simple concept, it is necessary to postulate from five to eight independent sequential steps. It has been suggested by Armitage and Doll (1957) that it is possible to arrive at the same equations by assumptions which, to them, are somewhat more plausible than a reaction requiring so many steps. This approach assumes that a somatic mutation occurs in a cell which confers on the cells of the resulting clone a selective advantage in division. After a number of cell divisions the daughter cells of this clone are considerably more numerous than they would be otherwise. At this point a second mutation can occur with a greatly increased probability. This idea would then only require two mutations but more might be involved. It is not possible at present to choose between this theory and a pure multistep one, but the difference is not great from a conceptual point of view.

As indicated previously, there are other factors which undoubtedly play a large part in the time course of the development of these diseases. For these reasons one should not expect to push the etiological approach to a very great accuracy. The chief reason for the exercise is to show that

the general picture as presented in Fig. 5 is compatible with the known facts.

In the light of the above discussion it almost seems unnecessary to point out that radiation accelerates some aspects of the aging process by the production of mutations in certain somatic cells. But since mutations are only one of the steps leading to each of the degenerative diseases, one would not expect a quantitative relation between the number of mutations and life expectancy. Thus if the number of mutations, as judged by the chromosome aberrations, were doubled, one should not expect the mortality rate to be doubled. This is true, since one of the assumptions involved in the simplified equations is that the rate constants are all about equal. If one of the rate constants were much larger than the others, then increasing it considerably would have little effect on the final result. Thus one would not expect an exact quantitative relation between number of mutations and disease incidence.

SUMMARY

Aging can be defined as an increasing probability for the individual to contract one of the degenerative diseases. It can be shown that most if not all these diseases probably involve one or more mutations in a somatic cell. Radiation is the most effective mutagenic agent known and also hastens the appearance of all of these diseases. Further, different kinds of radiation and radiation regimes shorten the life span of mice by an amount proportional to the number of mutations each of these treatments

produce. These facts greatly strengthen the mutation concept of aging and promote the idea that radiation accelerates the aging process.

The entire process of aging is considerably broader than the rather pragmatic definition adopted here. If one considers all the various age-related phenomena it is quite clear that many of them are quite unaffected by even rather large doses of radiation. Thus aging is a very complex biological process, and no single, simple theory will explain it, and no single agent could possibly be expected to affect all parts equally.

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FIGURE LEGENDS

Fig. 1. Chromosome aberrations in the anaphase figures in regenerating liver cells scored as a function of age in normal mice and in mice which received a large dose of x rays when 2 months old (from Stevenson and Curtis, 1961).

Fig. 2. 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 (from Crowley and Curtis, 1963).

Fig. 3. Chromosome aberrations in liver cells of dogs plotted as a function of age. The shaded area within the dotted lines indicates the limits, plotted on the same scales, of all strains of mice so far investigated by this method. The ages of the mongrel dogs was not accurately known so the line was drawn only through the points for the beagle dogs (from Curtis, Leith and Tilley, 1966).

Fig. 4. Chromosome aberrations in liver cells of the mouse strains indicated plotted as a function of age. The mice of strain C58 develop a fatal leukemia at about 10 months of age and are thus quite short-lived. The other two strains are long-lived. The genetic defect in strain C58 is obviously not apparent by the methods employed here (from Curtis, Tilley, Crowley and Fuller, 1966).

Fig. 5. A schematic representation of the aging process. A complex series of steps, each with its own probability or rate constant, is required for the final manifestation of each of the many degenerative diseases. One or more of the steps in each reaction almost certainly represents a mutation, but it is equally certain that they are not all mutations (from Curtis, in press).

Fig. 6. Log age specific death rate vs. age for the population indicated. Points for a 5th power function of time are also shown and it can be seen that they represent the data reasonably well, indicating that a representation such as shown in Fig. 5 is in accord with the known facts (from Curtis, in press).

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6