MOLECULAR BASIS OF AGEING

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ALL multicellular organisms age shortly after attaining reproductive ability. The cause of this universal phenomenon is a challenging biological problem. A large volume of data accumulated until early 1960s showed that several structural and functional changes occur both at the tissue and cellular levels as the organisms age. They are: (a) decrease in the number of post-mitotic cells; (b) decrease in the levels of hormones; (c) decrease in the permeability of membranes; (d) accumulation of age pigment; (e) decrease in the levels of enzymes even though a few enzymes do not undergo any change and a few increase in levels; (f) increase in the cross-linking and tensile strength of collagen; (g) decrease in antibody titre and immunocompetence, etc. These changes, being secondary in nature, do not explain the basic cause of ageing.

Observations such as: (a) fixed life span of all individuals within a species; (b) more or less a similar pattern of decline of various functions of all organisms after a short period of reproduction; (c) long life span of progeny of long-lived parents and short life span of progeny of short-lived parents and (d) similar life span of identical twins, indicate that the cause of ageing may have a genetic basis. However, factors like stress, nutrition, temperature and heredity may influence the ageing process that may account for the variability in the rate of ageing and life spans seen among individuals of a species. Three theories have been put forward that attempt to explain the basic cause of ageing at the level of genes.

The 'somatic mutation' theory of Szilard[1] states that mutations occur randomly and spontaneously, destroy genes and chromosomes in post-mitotic cells during the life span of an organism, and gradually increase its mutation load. This decreases the production of functional proteins, the number of post-mitotic cells and various functions of the organism. The theory is based on the data of Ross and Scott[2] who showed that rats exposed to whole body irradiation, that was too low to produce acute syndrome, died earlier than unirradiated controls. It was therefore believed that irradiation accelerates the ageing process. Later Curtis[3] reported that there is an inverse relationship between the dose of radiation given to mice and their longevity, and chromosomal aberrations like chromosome breaks and sister chromatid separation are greater in mice exposed to higher radiation. He proposed that ionizing radiations damage post-mitotic cells more than the pre-mitotic cells because the damaged cells are eliminated from the latter group and the undamaged cells continue to divide normally. Furthermore, the germ line cells are more resistant to chromosomal damage. Hence the normal perpetuation of the species is not affected.

These conclusions are, however, not supported by experimental data. The symptoms of senescence manifested in irradiated mice are not only different from those of normal unirradiated mice, but also the chemical mutagens like nitrogen mustard do not give a dose-effect relationship as that of radiation. Also, both diploid and haploid males of the moth, *Habrobracon*, have the same life span[4]. If mutagenic effect of radiation is the cause of ageing, then diploids should have longer life span as they have double the complement of genes that should withstand radiation effect better. In analogous experiments, Hoehn *et al*[5] and Thompson and Holliday[6] found that a certain proportion of human foetal fibroblasts in culture become tetraploid if they are treated by colchicine. However, both the tetraploids and normal diploids have the same division potential and life span. Even though it is postulated in the theory that germ cells are more resistant to radiation than somatic cells, the LD50 of man, mice and *Drosophila* is 450, 500 and 6400 roentgens, respectively. Further, although the DNA content in humans is 50- and

1200-fold greater than those of mice and *Drosophila*, respectively, they are more sensitive to ionizing radiation. One of the difficulties in testing this theory is that there is no accurate and reliable method of measuring somatic mutations, and the symptoms developed in irradiated individuals are different from those of natural ageing. Hence ionizing radiations and other mutagens may cause early death by increasing the frequency of cancer and other unknown factors.

Orgel[7] advanced the 'error' theory of ageing according to which errors occurring during information transfer processes like transcription and translation may cause accumulation of defective proteins and cause ageing of cells and the organism. Such errors include incorporation of inappropriate nucleotides into messenger RNAs during transcription such that triplet codons are altered leading to incorporation of inappropriate amino acids into proteins during translation. Likewise, inappropriate amino acids may be picked up by tRNAs during translation and incorporated into polypeptides leading to amino acid substitutions and error containing proteins. If such proteins belong to metabolic paths, the defect may be shortlived and not deleterious as their effects would be erased as soon as they are degraded. However, if such errors occur in the protein synthesising machinery itself like RNA polymerase and aminoacyl-tRNA synthetases, then the effect would be disastrous for the cell as all types of mRNAs and/or proteins synthesised would have errors. The error containing proteins would get amplified until the cell dies of 'error catastrophe'. Later, Orgel[8] proposed that such defects may not be transmitted from one generation to the other, and also that such defects do not occur in early age due to 'quality control' or error removing machinery. No experimental proof has been advanced so far for these predictions.

Several workers have used fibroblast cells in culture to test the 'error' theory[9-11]. They have suggested that cells in culture are potentially immortal and are initially uncommitted. However, after a certain period of population doublings, a few cells get irreversibly committed to senescence and death. They multiply for a period, and after a certain number of divisions called incubation period, they senesce and die. The uncommitted cells continue to divide. Kirkwood[11] suggests that commitment of cells in culture may be due to errors and that somatic cells have less ability to regulate errors. He argues that error is inherent in all processes of macromolecular information transfer and that "to sustain the prospect of further evolutionary change and to improve its chance of ultimate survival, an organism must make occasional copying errors". Though it is accepted that had the fidelity of information transfer system been absolute, there would have been no evolution, Hoffman[12] has suggested that the translation machinery is such that errors are not possible and hence 'error catastrophe' cannot occur. Hopfield[13] has suggested that errors are avoided in replication machinery due to proof reading and destruction of erroneous products. Kirkwood[14] has suggested that these mechanisms may be switched off at a later stage of development. However, we have instances of cell death during tail and gill resorption of the tadpole and formation of digits in birds and mammals. How are these cells, and not other cells, committed to senescence at such an early age?

Several workers, however, have failed to detect faulty proteins in old age. Studies of Kanungo and his co-workers[14-18] on the kinetic, antigenic properties and peptide maps of several enzymes purified from old rats have shown that they are similar to those of the young. Gershon and his co-workers have also shown that the antigenicity, K_m, K_i and electrophoretic mobility of superoxide dismutase purified from young and old rats are the same. Isoelectrofocussing also shows no differences in their electric charge[19-21]. However, differences in their specific activity and temperature sensitivity are seen. These differences are attributed to post-translational modifications of the protein. Similar observations were made for several enzymes of the free living nematode, *Turbatrix aceti*[22-24]. Thus, there seems to be no experimental proof to support the 'error' theory.

The 'gene regulation' theory was proposed by

Kanungo[25] and later extended as a model[26,27]. The theory takes into account the sequential changes in the expression of genes that occur from the beginning of the life span of an organism until the attainment of reproductive ability and completion of the growth period, and the gradual, and not random and sudden, decline in several functions seen during normal ageing. According to the theory, the regulatory mechanisms necessary for the normal functioning of the array of genes required for the maintenance of various functions including reproduction during adulthood gradually get destabilised as the organism is unable to maintain the levels of factors that are necessary for induction and/or repression of these genes. As a result of reproduction and other processes associated with growth, certain factors get depleted which the organism is unable to replenish, and certain factors accumulate which it is unable to get rid of. Hence there is a gradual decline in the homeostatic functioning of genes required for the maintenance of adulthood. Also, as a result of accumulation of certain factors and depletion of others certain undesirable and harmful genes get expressed which may result in the appearance of cancer and autoimmune diseases. The following experimental findings support the theory.

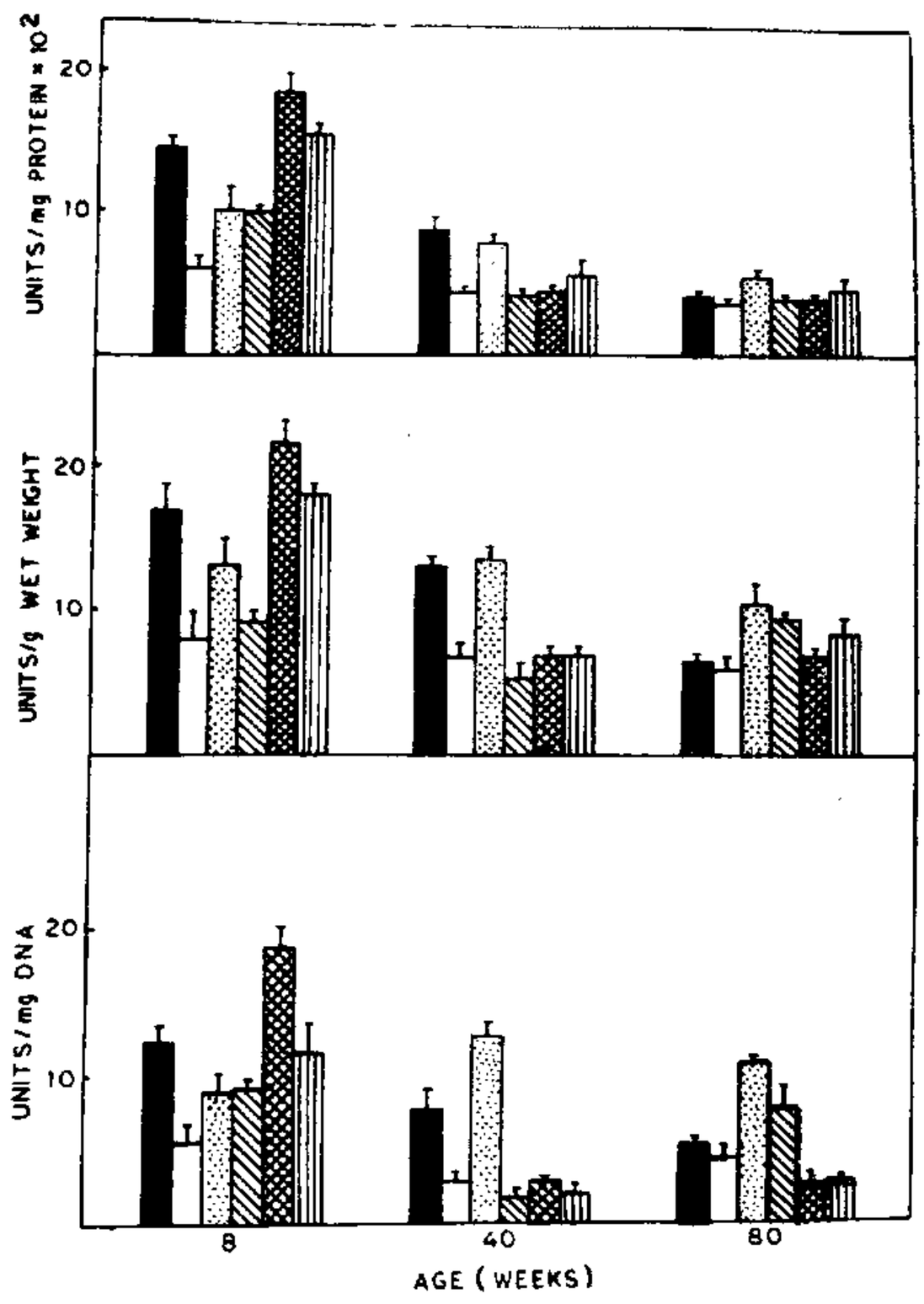

Figure 1. Effects of estradiol and testosterone on cholineacetyl-transferase activity of the cerebral hemisphere of female rats of various ages. ■, control; □, ovariectomized; ▩ and ▥, 17β-estradiol 10 and 100 μg respectively; ▤ and ▨, testosterone 10 and 100 μg, respectively.

(i) Several enzymes belonging to different species and organs decrease in activity as a function of age. Certain enzymes, however, do not show any change and a few increase with age.

(ii) The changes in enzyme levels occurring during ageing are reversible. The levels of certain enzymes which decrease in old age can be raised and brought back to adult levels by administration of steroid hormones, *e.g.* cholineacetyltransferase[18] (figure 1), pyruvate kinase[17] and tyrosine aminotransferase[28]. Since steroid hormones produce their effects by acting at the level of genes, the decrease in enzyme levels seen in old age may only be due to a decrease in the expression of respective genes.

(iii) Studies on kinetic, antigenic properties and isoelectrofocussing of enzymes purified from young and old animals of various species do not show any differences. Also, peptide maps of proteins purified from young and old proteins are the same[29]. Thus, no differences in the primary structures of proteins occur as organisms age. Hence, the primary structure of genes coding for these proteins do not change with age.

(iv) The isoenzymes of several enzymes show sequential appearance and disappearance during ageing *e.g.* alanine aminotransferase[16] of rats (figure 2) alcohol dehydrogenase and hexose-P-isomerase of

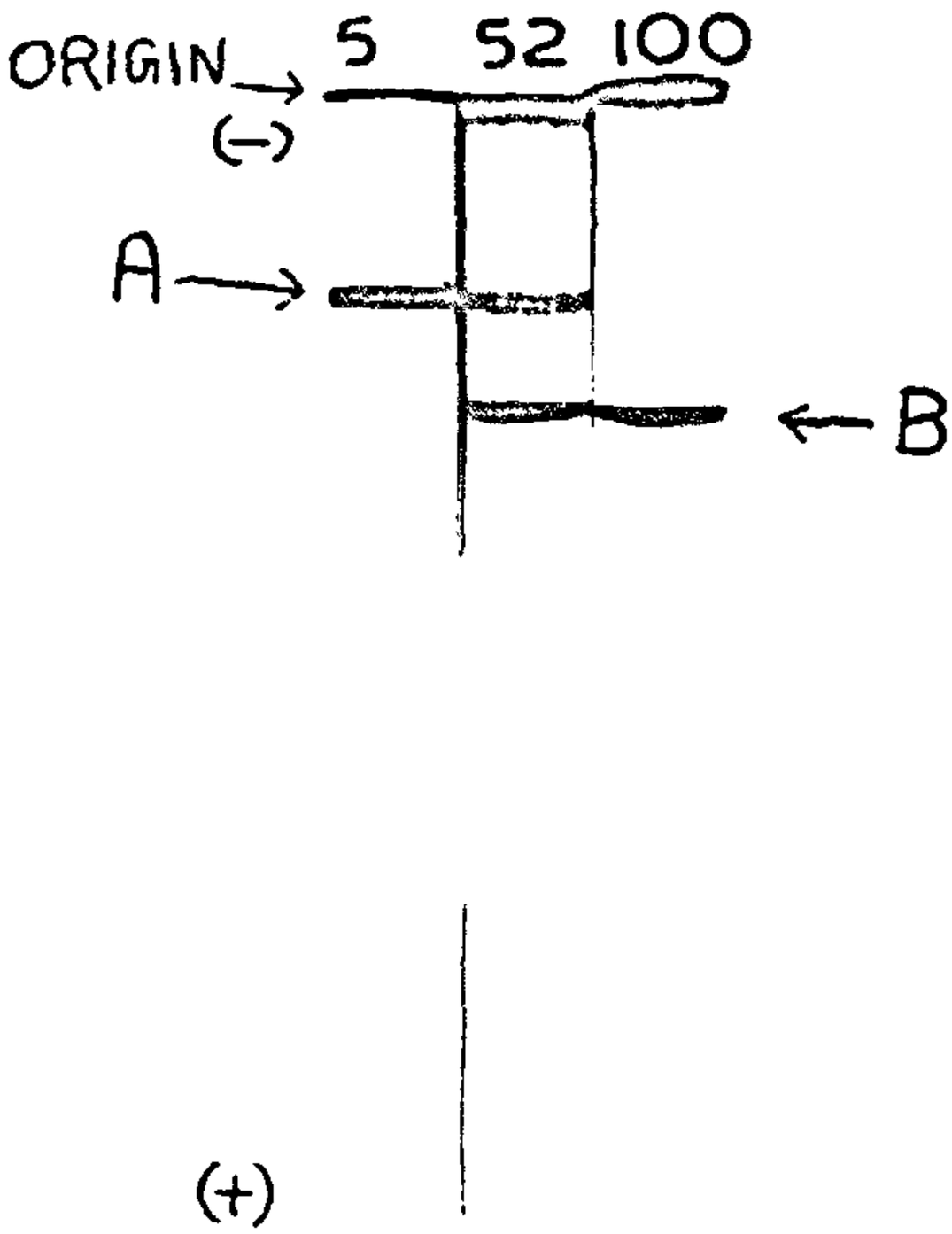

Figure 2. Polyacrylamide gel (7.5%) electrophoresis of soluble alanine aminotransferase of the liver of immature (5-week), adult (52-week) and old (100-week) female rats. A, sAAT-A ; B, sAAT-B. 50 μg of protein was loaded.

Drosophila[30,31]. Different subunits of these multimeric enzymes are coded by different genes and these genes get expressed in a sequential manner, evidently modulated by certain factors that may appear at specific stages of the life span.

The above data show that alterations that occur in the levels of enzymes may only be due to alterations in the expression of genes and not due to any structural changes. Since the genes (DNA) are complexed with chromosomal proteins, histones and nonhistone chromosomal proteins (NHCP), their expression may be modulated by various types of chemical modifications that occur in these proteins such as phosphorylation, acetylation, methylation and ADP-ribosylation. Hence studies on these modifications and their effects on transcription were carried out as a function of age of rats[31-33]. Some of the important findings are:

(a) Acetylation is correlated with transcription. It was found that incorporation of labelled acetate into histones of the chromatin of the brain decreases during ageing. Concomitantly, incorporation of ^{3}H-UMP into RNA also decreases.

(b) Sodium butyrate which inhibits deacetylase, hyperacetylates histones. This effect is high in the young and gradually decreases with age. Also, stimulation of transcription caused by hyperacetylation in the young is not observed in the old.

(c) 17β -Estradiol also causes hyperacetylation of histones and stimulates transcription in isolated nuclei of the brain of young and adult rats, but this effect is not observed in old rats.

These findings gave strong indication that conformational changes occur in the chromatin, particularly of post-mitotic cells, as a function of age. If it is so, then the digestibility of the DNA in the chromatin should show differences with age as the DNA is wound around histone octamers. Hence the digestion of DNA of the chromatin of the brain of young and old rats was done by two endonucleases, micrococcal nuclease (MCN) that cuts DNA in the inter-nucleosomal region generating 200 bp fragments and its multiples, and DNase I that cuts DNA at 10 bp fragments and its multiples[34]. Since the basic structure of chromatin is made up nucleosome which has eight core histones around which DNA is coiled, chemical modifications of histones were also carried out before digesting the DNA by the two endonucleases. The studies show that:

(i) The digestibility of chromatin DNA by DNase I decreases with age as seen by both kinetics of digestion and production of 10 bp fragments and its multiples. In old rats, 10 and 20 bp fragments are far less than in young rats. Butyrate stimulates digestion by DNase I due to hyperacetylation. However, this effect also decreases with age (figure 3).

(ii) The digestibility of DNA by MCN does not show any change with age. Eventhough butyrate stimulates digestion, no difference is seen in the digestion pattern as a function of age.

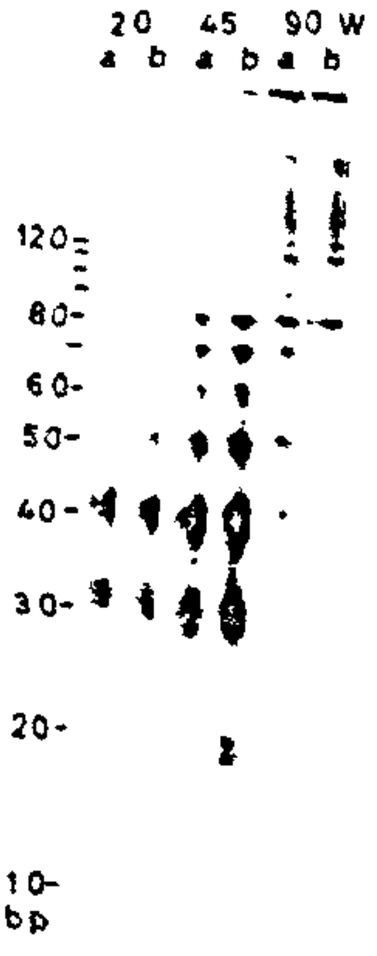

Figure 3. Denaturing polyacrylamide gel (12%) electrophoresis of DNase I-digests of nuclei (a) and chromatin (b) of female rats of various ages. 10 μg DNA was loaded in each lane.

These findings show that conformational changes occur in chromatin of post-mitotic cells such as neurons and muscle cells so that the sites for acetylation and other chemical modifications of chromosomal proteins are not available for the respective enzymes. Such changes also may make endonuclease sites on DNA inaccessible to the two enzymes studied. It is possible that these modifications in the chromatin of post-mitotic cells bring about a gradual change in the conformation of chromatin resulting in a gradual alteration in the expression of genes and cause senescence (figure 4).

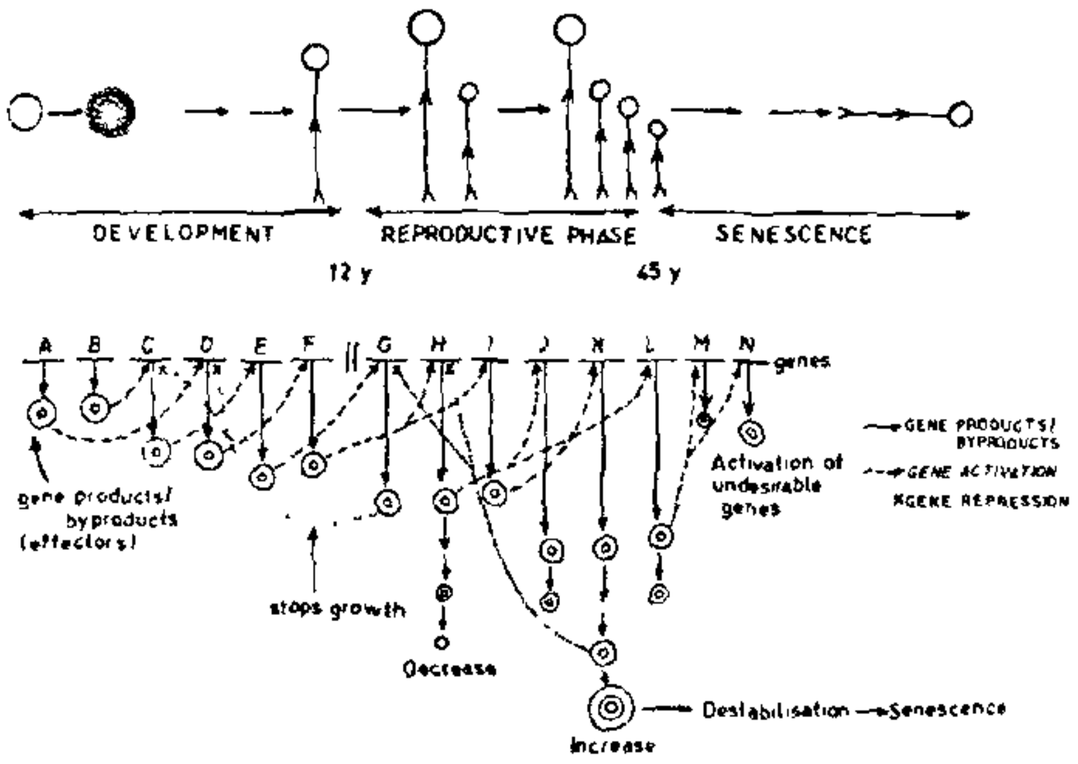

Figure 4. Model for ageing (modified from Kanungo 1975).

ACKNOWLEDGEMENTS

Generous grants from the Nuffield Foundation (London), PL-480, Department of Science & Technology and University Grants Commission to M.S.K. are appreciated.

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ANNOUNCEMENTS

ENVIRONMENTAL SOCIETY HONOURS DR. SWAMINATHAN

Dr. M. S. Swaminathan, Director, International Rice Research Institute, Manila has been awarded the Prof. R. Misra medal by the Indian Environmental Society (I.E.S.).

The medal instituted in 1980 would be presented to Dr. Swaminathan on September 10, according to Mr. Desh Bandhu, President of I.E.S.

NATIONAL SEMINAR ON DRAUGHT ANIMAL POWER

National Seminar on Draught Animal Power (DAP) System will be held at Bangalore from 16–18 July 1982. The Seminar is sponsored by the Commission on Additional Sources of Energy (CASE), Department of Science and Technology, Government of India and is organised by the Indian Institute of Management, Bangalore. The main objectives of the Seminar would be to: (1) Review the state of the art of the various aspects of the DAP System; (2) Identify the problems in the DAP System, which have led to the low level of utilization and productivity; and (3) Evolve proposals for increasing its utilization and efficiency.

Further particulars may be had from: Prof. K. L. K. Rao, Co-ordinator, Seminar on DAP, Indian Institute of Management, 33 Langford Road, Bangalore 560 027.

VIVIDHAXI AUDYOGIK SAMSHODHAN VIKAS KENDRA (VASVIK) AWARD 1980

Prof. U. R. Rao, Director of Satellite Centre, Indian Space Research Organisation (ISRO) Bangalore has been awarded the VASVIK 1980 award for electronic sciences. The award carries a cash prize of Rs. 25,000/- and gold medal.

THIRTEENTH INTERNATIONAL CONGRESS OF MICROBIOLOGY

The Thirteenth International Congress of Microbiology* will be held at J. B. Hynes Auditorium, Boston, Mass, USA, during August 8–13, 1982.

Main topics of the Congress are as follows:

BACTERIOLOGY DIVISION – Bacterial Taxonomy, Systematics and Evaluation; General Bacteriology and Ecology; Genetics and Molecular Biology; Infections and Pathogenesis; Cell Growth and Division; Physiology, Metabolism and Structure Biotechnology; Miscellaenous Topics: Training Microbiologists, Quality Control and Proficiency Testing, Use of Computers.

MYCOLOGY DIVISION – Mechanisms of Pathogenesis; Fermentation; Fungal Toxins; Ethanol Production by Yeasts; Enzyme Regulation in Fungi; Immunological Aspects of Systemic Mycoses; Fungal Genetics.

VIROLOGY PROGRAMME – Genetic Control of DNA; DNA Packaging *In Vitro* and *In Vivo*; New Phages and Comparative Studies; The Lysogenic Description.

*Convened by the International Union of Microbiological Societies.