SOME BIOMEDICAL INDICATORS IN CHRONOGENETICS

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All living beings are variously conditioned by both continuous and repetitive chronological phenomena.
The greater biochemical complexity afforded by the genome of higher organisms contributes to increase their independence from environmental chronological phenomena (= increased homeostasis). Genetic variability (originated by mutations and required for evolutionary adaptation) extends its influence on the biochemical mechanisms of homeostasis.
Phenotypical variability, if prevailing environmental conditions are constant, is based on genotypical variability. Thus the variability observed in the duration of homeostatic mechanisms must be genotypical.
In current chronogenetical theory, Gedda and Brenci's concept of the Ergon/Chronon System provides the explanation for the observed variability in the duration of homeostatic phenomena; in this concept, the differential stability of genic information explains the variation of patterns of senescence in different individuals. From the viewpoint of the continuity of life, senescence must be considered as "physiological", but its individual, especially initial degenerative manifestations are generally considered as pathological. The fundamental applications of chronogenetics to the human species are to be aimed in this direction.
Individual phenomena of senescence may be more or less linear; the pattern of the respective curves may be common to the entire species, or else it may afford varying degrees of variability. The ascertainment of the respective curves (for which some indicators may serve as examples) is a prerequisite for the application of chronogenetics to the individual, framing him within his genealogy and providing great potential developments for preventive medicine.

Chronogenetics is a recent, rapidly developing branch of biomedical science. It studies the relationship between heredity and chronological phenomena in living beings.

Time is marked by both continuous and repetitive phenomena. The degradation of complex chemical structures, leading to increase of entropy, appears to be continuous. The cyclic nature of most phenomena in our solar system is obviously repetitive.

Life also exhibits both continuity and repetition. The continuity of life flows through time, ever since it first appeared on earth, through millennia of reproduction, mutation and evolution. The principles of coupled reactions and enzymatic control, under the self-perpetuating rule of nucleic acids, enable living beings to increase their biochemical complexity during development, only apparently contrasting the second law of thermodynamics. Thus the life of every living being, and most strikingly that of higher plants and metazoa, manages to convert the continuous flow of energy through time into a definite curved pattern. The

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sequence of such patterns in successive generations, through birth, development, reproduction and death, becomes a repetitive cycle.

The repetitive cycle of individual life may be more or less closely affected by the repetitive cycles of the environmental conditions. Seasonal changes, night and day, heat and cold, the moon cycle, ebb and tide, represent so many repetitive changes variously affecting life.

The increasing complexity of higher organisms has come to include highly sophisticated biochemical mechanisms to compensate environmental variability, resulting in increased homeostasis.

Homeothermy is an obvious example of how living beings can manage to compensate cyclic environmental changes, thus gaining some degree of freedom from repetitive time. The independence of the human species from repetitive time may find perhaps the best instance in the continuity of the... mating season. Although the woman's menstrual cycle tends to approach the frequency of the lunar month, it is an independent phenomenon continuing throughout the year.

Yet, this biochemical complexity, whose information is imprinted in zygotic DNA, is dependent on the stability of such information.

If informational stability were absolute, individuals with separate somatic and gametic lines would tend to be immortal, unless exogenous noxae or programmed death intervened. This would perhaps satisfy their egos, but would be incompatible with the laws of life in general. The usefulness of an individual for his species passes its peak when an appropriate number of descendants have been readied for independent life. After that moment, the individual is expendable or, even better, a growing burden.

The traditional respect and appreciation expressed for the aged in the human species is an exception, probably related in part to the value of accumulated experience. The fact that recent generations tend to show less concern for the aged (apart from their own self-preservation and a sense of moral — not biological — obligation) may be related to the growing transfer of experience and knowledge from the mind of the elders to other supports.

Apart from this human exception, aging and death seem to be closely related to the time required for the generation and upbringing of descendants to the point of self-supporting capacity. Incidentally, this concept may be usefully applied in comparative studies of mean life span. The interdependence of reproductive age and mean life span has often been investigated, but the fact that the newborn offspring in higher species is practically incapable of independent life has been hardly considered. Revised tables, considering such variables as mean age at reproduction, mean number of offspring per parent for species survival, and mean duration of parental care, may reflect more fully the requirements of mean life span in different species. Such may be the case for men and for women.

Differences between species as to mean life span thus acquire obvious evolutionary value, but the mechanisms are still largely unknown. The differences between such relatively similar species of mammals as mice, dogs, horses, and men appear hardly explainable, for instance, in terms of different degradation rates of biochemically similar substances, as pointed out by Walford (1969). It seems easier to find an explanation either in a different programming of life span or in a differential stability of genic information or in both.

A discussion of differences in life span brings us to consider the interrelated phenomena of aging, senescence and death. Aging and senescence are often used interchangeably. It seems more appropriate to distinguish the two concepts: aging may be used to mean simply that the organism grows older, while the concept of senescence takes on the added meaning

of gradual deterioration, both structurally and metabolically. In this view, aging is the growing measure of time in the individual's life, while senescence represents the variable consequences of aging.

Variability (and its source, mutation) being the requirement for adaptation and evolution, the general pattern of senescence in each species offers an almost infinite variety in the individuals, especially in higher organisms. Each variation may be more or less " fit " in any evironmental condition.

Since man has attained (often artificially) comparative independence from environmental conditions, most deviations from " normal " fitness are considered as pathological. Thus the decreasing fitness of senescence, although physiological from the viewpoint of the continuity of life, is often considered as pathological in the individual.

If aging and senescence (and death) were to follow relatively parallel patterns (as seems to be the case in lower organisms) it would be easier to assume that one basic mechanism were responsible for senescence and death. The relative constancy of life span in certain species suggests that a definitely programmed death schedule may be involved (probably by regulation of gene actions, as is the case for the programmed succession of developmental stages). The programmed involution and death of tissues and organs that we observe in the successive stages of insect development could easily be extended to involution and death affecting the final, adult form as well.

But in higher organisms the picture is less clear. Senescence exhibits a great measure of phenotypical variability. Now, phenotypical variability, if prevailing environmental conditions tend to be constant, may be originated only by genotypical variability, and vice-versa.

Let us consider an example in the human species. The gradual changes in environmental conditions may act upon a genotypically different population over the millennia and in part over the centuries, inducing changes in life span that may be partly of environmental and partly of genetic origin. But changes in evironmental conditions over ten or twenty years act upon populations that tend to be genetically superimposable.

In Fig. 1 (Comfort 1965) the human survival curves for females in different environmental conditions are compared. It is striking to see how different survival curves are when comparing the stone age (no. 10) to modern times. The radical changes in health conditions, especially for children, are illustrated by comparing curves 9 and 8 (India and Mexico in the twenties) with 1 and 2 (New Zealand and U.S. in the late thirties). Even comparison between curves 6 and 2 (U.S. Whites, 1900 and 1940 respectively) shows how much the 50 per cent survival point has been moved to the right with biomedical and social progress.

Yet biologists cannot fail to find at least equally striking the fact that the *maximum* life span, estimated at 50 years in the stone age, has not been displaced in the current century from its limit of about 100 years. The relative constancy of this upper limit seems to suggest the existence of a programmed death schedule, which especially in past centuries was hardly ever reached because of the excess of exogenous causes of death.

In fact Walford (1969) has drawn, in a similar comparison (Fig. 2), a curve for ancient Rome (A) and one for the U.S., 1940 (B), both tending to the same upper limit of about 100 years, and a third curve, properly identified as hypothetical, assuming an extension of maximum age. We do know that a number of factors may affect life span: infections, irradiation, accidents, may shorten life; yet prolongation of life beyond its normal upper limit appears to be possible (from experimental data) under the influence of one factor only, i.e., caloric undernutrition in the developmental stages. Apart from this exception, hardly foreseen as

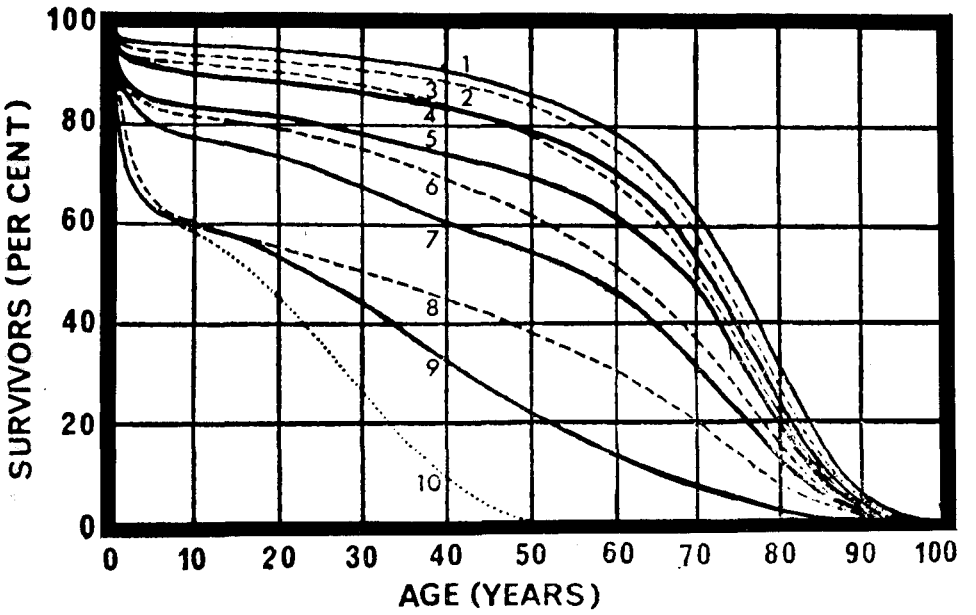

Fig. 1. Human survival curves, for females, reflecting the effect of improvement in living conditions. [*After Comfort 1965*].

1 New Zealand, 1934-38
2 U.S. Whites, 1939-41
3 U.S. Whites, 1929-31
4 England and Wales, 1930-32
5 Italy, 1930-32
6 U.S. Whites, 1900-02
7 Japan, 1926-30
8 Mexico, 1930
9 British India, 1921-30
10 Stone Age Man (guesswork)

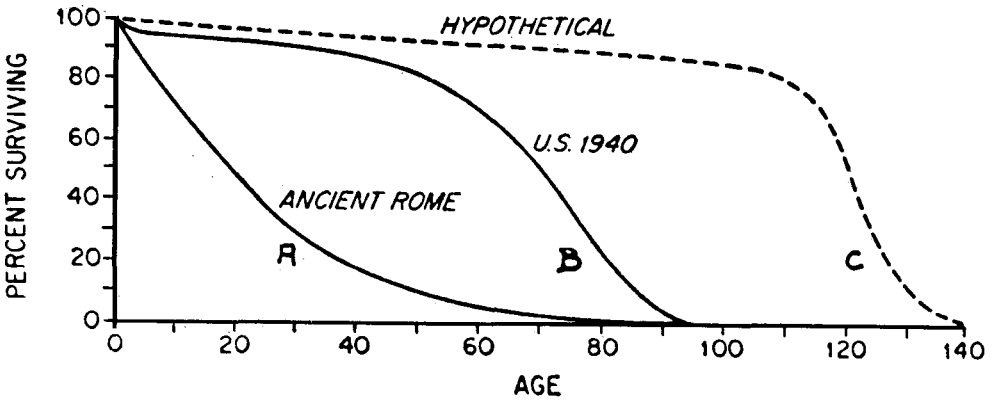

Fig. 2. Survival curves for ancient Rome, the U.S. as of 1940, and a "hypothetical" population in which aging has been retarded. [*After Walford 1969*].

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applied to the human species, it appears that human life is controlled by one relatively constant upper limit and an extreme variety of individual death-causing factors operating at intermediate ages.

The factor reponsible for this apparent upper limit may be quite different from all others. Many general theories have been proposed for senescence and death, and we shall not even try to review them. It seems that each theory has at least partial validity, and this appears quite easily explained. For instance, the different situations concerning " perennial ", stable, or high-turnover tissues and structures seem to involve different mechanisms of senescence. Let us mention Gedda and Brenci's Ergon/Chronon system, already formulated in several publications and in the opening paper of this session, relating variability in the pattern of senescence to differential stability of genic information. This concept, consistent with many experimental facts and compatible with other " theories of aging " such as Walford's immunological one, does not seem to encompass directly such phenomena of senescence as collagen deterioration.

Collagen changes represent one of the most dependable indicators of aging. Collagen fibers being a relatively stable biological structure, the influence of genic stability on its deterioration is to be considered secondary at most (metabolic homeostatic mechanisms, such as production of inhibitors of cross-linking agents, can easily be considered in this light). The remoteness of the cause-effect relationship between genic information and collagen trophism (as in the Ergon/Chronon system concept), on the other hand, provides an explanation for the finding that collagen " aging " seems to be independent of irradiation — a finding hard to explain in some other theory.

Wear and tear (or cross-linking, or decreases in solubility, etc.) of long-lasting structures are certainly part of the total phenomenon of senescence; degradative changes in such structures tend to be linear with time, causing increased disability but without definite onset of a disease.

Such linear degradation of permanent structures can be envisaged as related to the maximum limit of life span, being " programmed " to last only for a definite length of time. The alternative form of programming of death would be, of course, an active rather than passive event, such as the repression or derepression of one or more operons charged with termination of life.

The popular expression " died of natural death " or " died of old age ", applied to old people who die without apparent cause, seems to refer to just such a situation. It is of course pure chance that, just a week before this Congress started in Rome, the papers reported the death of " Rome's Granny ", our oldest citizen, aged 104, with the following words: " Up to the end she enjoyed perfect good health; she suddenly died of natural death. "

But even if we could postulate the existence of a programmed upper limit of life span, the fact remains that most individuals do not attain such a limit. Thus we come to concentrate our attention on mean, rather than maximum, life span.

As already illustrated, our mean life span has been consistently elevated in recent years, thanks to the improvement of environmental conditions. But mean life span is only a statistical concept, resulting from millions of individual life spans, each characterized by different limiting factors. Now, the relative standardization of environmental conditions having failed to standardize life span, we must go back to the statement that " phenotypical variability, if prevailing environmental conditions tend to be constant, may be originated only by genotypical variability ."

Thus genotypical traits, already mentioned as responsible for interspecific differences as to mean life span, are even more obviously responsible for intraspecific variance.

By the trial and error mechanism of evolution, the human machine has come to its present form and function, apparently designed to last for a maximum span of about a century. But trial and error in the myriad components of this complex machine continues, and each component may reveal different degrees of stability according to differences in manufacturing specifications.

The manufacturing specifications in this example correspond to the information imprinted in the genes, and we refer to the already mentioned Ergon/Chronon theory as the currently most complete general interpretation on the genetic mechanism of chronological variability.

Much as general and comparative studies on mean life span, survival rates and incidence of death causes can help us to understand the processes of senescence, whenever we turn to individual cases, especially if clinical cases, we come to grips with the separate aspects of such phenomena. The fact that women generally lose their hair only in extreme old age is no help in a precociuos case. Arteriosclerosis generally becomes a clinical entity after age 60, but this is no comfort and hardly clinically useful for an individual who may suffer from it at 40.

At this point it becomes important to be able to assess, for each separate phenomenon of senescence,

(*a*) whether or not a given clinical curve exists in the human species;

(*b*) the degree of variability, as to pattern of the curve, between individuals and between ages of onset.

Only then will prediction values be obtainable for each phenomenon from biomedical indicators of forthcoming manifestations of senescence.

But what kind of a curve should we try to construct, and what kind of biomedical indicators are we supposed to seek?

In Fig. 3 (Ciba Foundation 1959) we see the often-cited tables of the percentage of individuals with lesions, and of the distribution of ages of onset, for five pathologic forms in man and, represented on the same scale, for four pathologic forms in the rat. The per cent incidence and the probability of onset for the same four entities, plus a fifth one, in the rat, is represented in Figs. 4 and 5 (Simms and Berg 1957). The distribution in both species is compared in order to evidence certain similarities, which are useful when trying to apply by analogy to human senescence processes the results of experiments carried out in other species.

Focusing now our attention on the human part of Fig. 3, it is clear that these curves represent either percentage of affected persons or distribution of the ages of onset in a population, but they do not represent the sequence of events occurring in the individual.

Whenever a phenomenon appears to be linear, the difference between curves of incidence and curves of severity is of course less important, unless threshold effects intervene. For example, the levels of certain natural antibodies in man have long been known to decline with age (Thomsen and Kettel 1929). The decline tends to be linear (as confirmed by our own twin studies). Now, individual antibody levels at any given age may vary, and this variability is largely under genetic control. Our most recent findings, applying Holzinger's formula to twin data on alpha-isoagglutinin titrations, indicate that genetic control of titer is 0.87 at 10 years of age and 0.64 at 60 years. The decline we observed in genetic control is not surprising: since we are dealing with an immune reaction to exogenous, variable antigens, the variability of the environmental component tends to accumulate over the years.

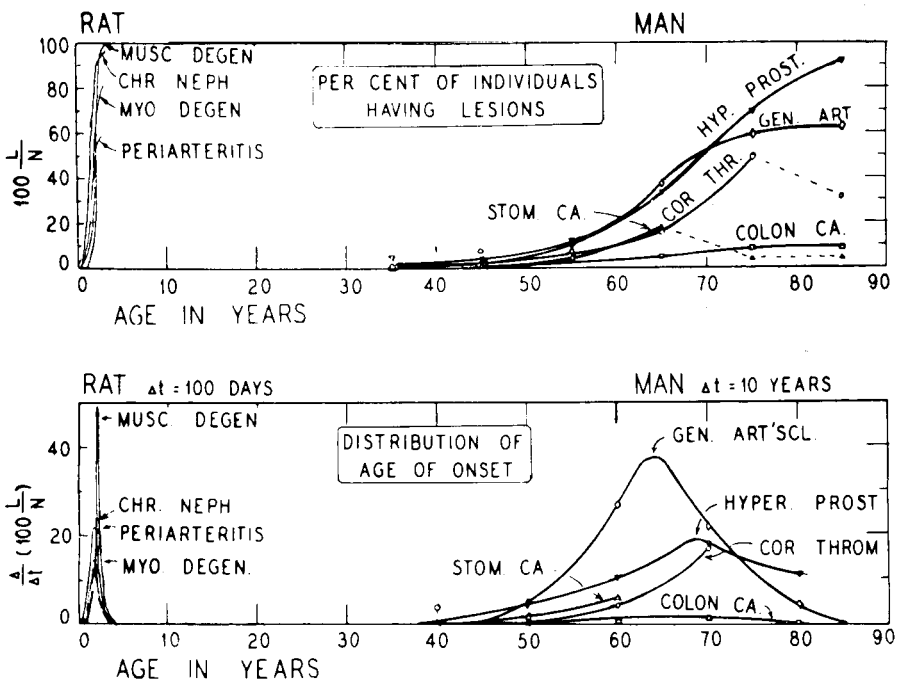

Fig. 3. Percentage of individuals with lesions, and distribution of the ages of onset, for five pathological forms in man and, represented on the same scale, for four pathological forms in the rat. [*After Ciba Foundation 1959*].

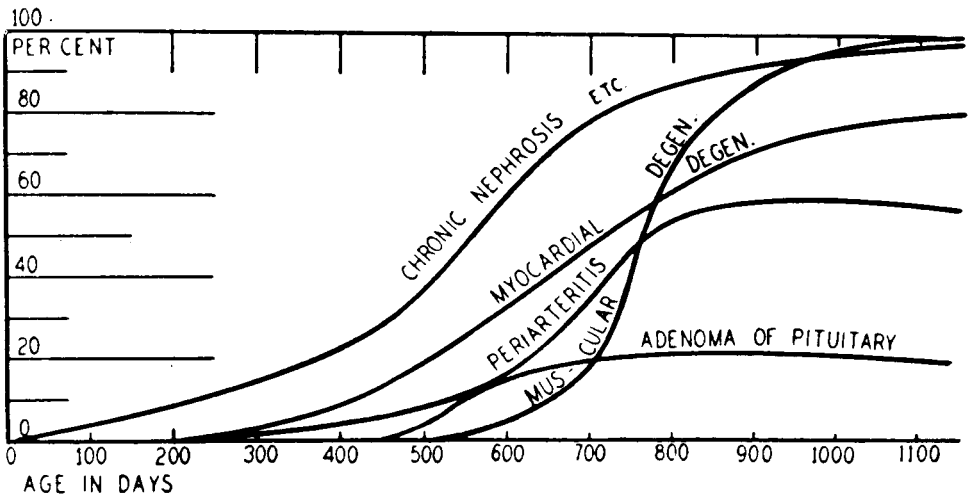

Fig. 4. Percentage of individuals with lesions with increasing age in rats. [*After Simms and Berg 1957*].

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In any case, the finding of a high degree of genetic conditioning in the level of an immune reaction is quite interesting. Since the level of this reaction shows a linear decline with age, those who are genetically situated at a relatively low initial level can be plotted to reach dangerously lower levels at a relatively early age.

That immune mechanisms are involved in homeostasis is obvious (even, we trust, for those who may choose not to accept in its entirety Walford's "immunologic theory of aging").

Whether or not an early decline in one aspect of the immune response in an indicator of the general condition of the immune system (and related to the total state of health of the individual) has still to be definitely assessed, and we have only started to work in this area.

Going back to linear or nonlinear degradation patterns, we can observe another one (the ability to distinguish objects of given size at short distances) which tends to approach linearity of decline after abandoning a plateau around age 30 (Fig. 6, Heron and Chown 1967). In this case an early decline may be considered as an indicator of " anticipation " in the onset of a decline that is expected to proceed with a linear pattern. But even in this case it is not quite clear from the available literature whether an early onset initiates a linear pattern that is parallel to that of normal or late onset. If early onset is followed by a linear but steeper-than-normal decline, than predictive values are quite different for equal levels at different ages.

Linearity of decline with growing age seems to be the rule, and we can use some examples drawn from quite a different living species: the house fly (Figs. 7 and 8, Rockstein and Baker 1972). Although exhibiting linear decline patterns in their late phases, the curved patterns of most of the substances considered extend quite far into the adult age. If similar curves were analyzed for the human species, looking for indicators of potential homeostatic failure, the predictive value of a low level of activity in an enzyme would be much less clear if it were not known to what phase of a curved pattern it belonged.

Of course these are, once more, mean curves, and we do not know what their respective patterns would be if they were slightly or grossly abnormal.

Patterns of concentration or activity levels in humans are generally more intensely studied in pathological cases, but relatively few studies are available on prepathological patterns. Yet biomedical indicators can only be really valuable if they are appraised at a preclinical stage.

A good example is represented by phenylketonuria. The genetic nature of the disease had long been known, and no therapy was possible. At present, when abortion is easily suggested for genetic disease, phenylketonuria would have been included in the list of abortion-justifying disease, once its " biomedical indicator " had been found.

The discovery that a special diet will abolish the pathological consequences of the defective gene only if the " indicator " is revealed early enough (i.e., with clear chronogenetical implications) has completely changed the situation.

We can easily assume that the critical period for the phenylketonuria gene may duplicate the developmental situation represented by trehalose concentration in the house fly in Fig. 8.

Only if defective situations are detected at a prepathological level may preventive measures generally be taken.

A further example of a prepathological indicator may be found in the Staub Test for diabetes.

At present some indicator seems to be available for muscular dystrophy, even if it is still impossible to prevent the final occurrence of the pathological form. In this case (as in the

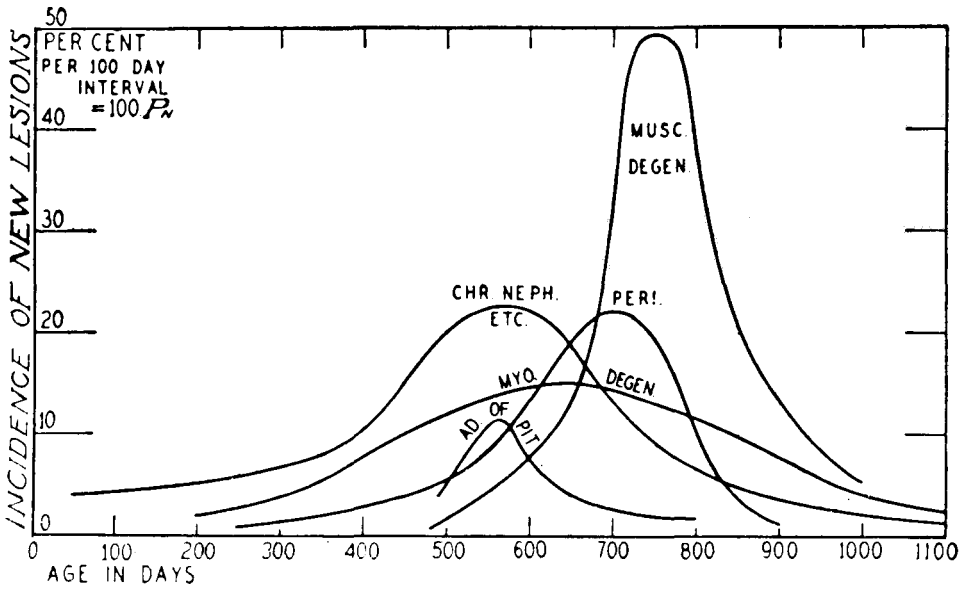

Fig. 5. Probability of onset of five pathological entities in rats with increasing age. [*After Simms and Berg 1957*].

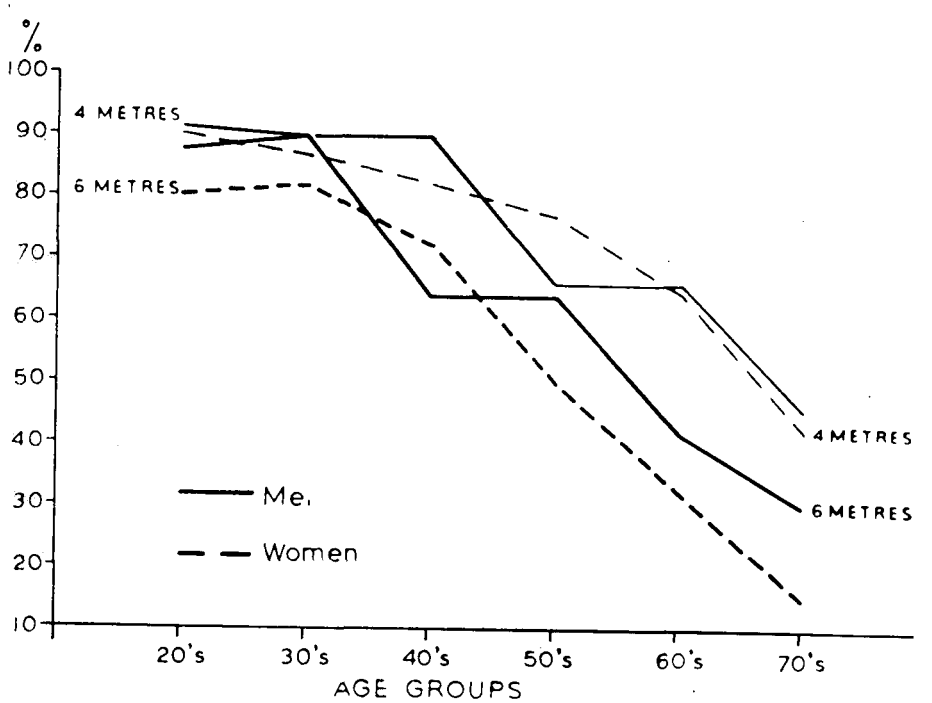

Fig. 6. Ability to distinguish object of given size at short distance. [*After Heron and Chown 1967*].

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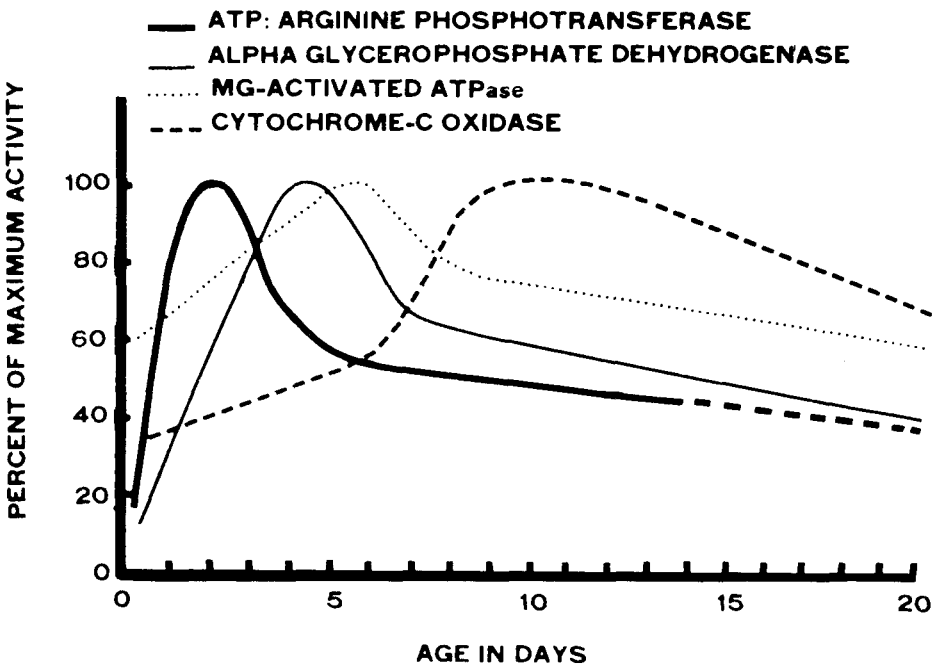

Fig. 7. Changes in the activity of four enzymes with increasing age in the house fly. [*After Rockstein and Baker 1972*].

case of diabetes) predictive value can be gained from the age of appearance of the indicator, since the time sequence of pathological manifestations in muscular dystrophy is much steeper in cases of early onset.

An indication of a similar situation may be found in a further illustration concerning muscular degeneration in rats (Fig. 9, Simms and Berg 1957). Here we see the time lag between occurrence of first " indicator " symptoms and occurrence of severe lesions. As can be seen, the lag is always short, but it is even shorter when onset is earlier.

Similar tables would be very useful in human senescence studies.

Some practical, even if presently rather wishful conclusion can be reached at this point:

(1) The widest possible number of age-dependent patterns in biological data should be collected, comparatively, on normal subjects and on families of index cases with genetic diseases, trying to detect the first indicators of prepathological situations;

(2) Molecular genetics by now supports the statement that a genetic disease is always related to biochemical abnormalities. The example of phenylketonuria should encourage us to seek, for each genetic condition, some biomedical indicator of impending morphofunctional degeneration;

(3) Much ingenuity will obviously be required in the effort to identify, for each biochemical defect, and exogenous balancing factor to restore homeostasis. At that point the availability of chronogenetical indicators will reveal their fundamental role in preventive medicine.

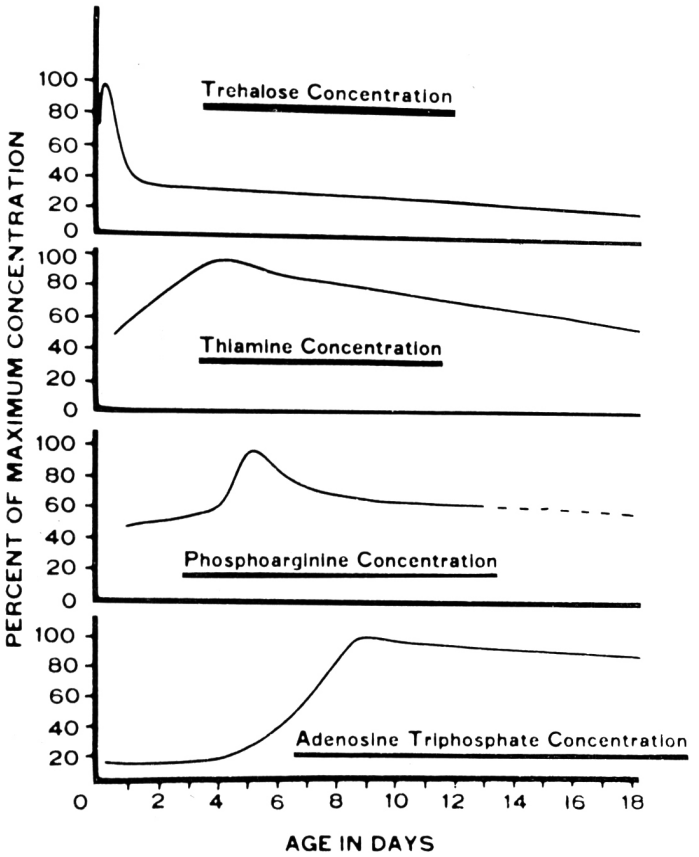

Fig. 8. Changes in the concentration of four substances with increasing age in the house fly. [*After Rockstein and Baker 1972*].

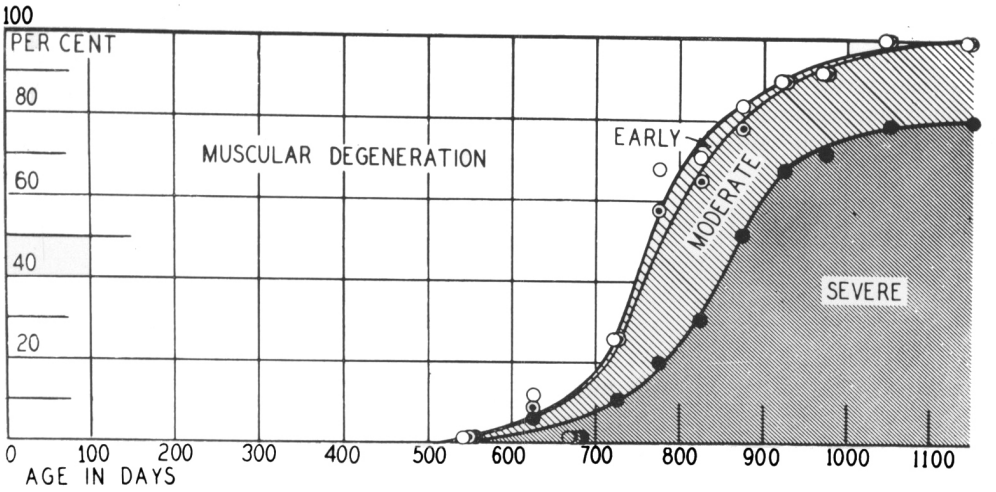

Fig. 9. Severity of disease (muscular degeneration) in rats sacrificed at different ages. [*After Simms and Berg 1957*].

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