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BIOMARKERS OF AGING

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Abstract — This article presents a conceptual discussion of some aspects involved in biomarkers of aging. A biomarker of aging is a biological parameter of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some late age than will chronological age. The reasons for undertaking biomarker research, criteria for putative biomarkers, measurement and assessment of putative biomarkers, and the new initiative by the National Institute on Aging in biomarker research are discussed.

Key Words: physiological age, chronological age, predictors of functional capability, species longevity, age-associated pathologies, biomarkers of aging, interventions in aging, validity of biomarkers

INTRODUCTION

AGING IS a complex biological phenomenon that has been observed in virtually all multicellular organisms that have been studied. The phenomenon is characterized by a progressive and irreversible reduced capacity of the organism to withstand the stresses of everyday life. Although the underlying molecular–genetic mechanisms which must ultimately be responsible for the species specific rates of aging are poorly understood, the grosser physical and physiological manifestations of aging process are well characterized in many species, including the human. It is the differences in the manifestations of aging processes both between species and within the same species that lend credence to the investigation of biomarkers of aging. The basic premise underlying the concept of biological markers of aging is that chronological age per se is not necessarily a good predictor of physiological or functional age, particularly at later stages in the life span of an individual. When one examines the life span as well as physiological capabilities of various species, even those of the same phyolgenetic class or order, there are striking differences, although one assumes that the same basic biological mechanisms we call aging are operational (see Table 1 for examples of *captive* maximum mammalian species longevity by order). A word of caution in interpretation of reports on maximum species longevity is necessary. First, such reports are often based on suspect validation of the data and are therefore of little or no value for biogerontological research. Second, even if

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data on maximum longevity for species were obtained under optimal controlled environmental conditions, the data would, by definition, represent a single, potentially anomalous, value which would not likely be representative of the environmental conditions for that species in the wild. With regard to the particular data set presented in Table 1, these maximum longevities for various species are taken from zoo records around the world as compiled by Jones (personal communication, November 1980). There is no information as to environmental conditions or cause of death in this compilation. It is clear, however, that there are factors other than just the passage of time which govern rates of aging for various processes, both at the inter- and intraspecies level. The products of these physiological, biochemical, and/or molecular–genetic mechanisms and the predictive relationships among these products as they are related to age is the essence of biomarker research.

In addition, there is a pragmatic interest in establishing reproducible scientific criteria by which one may assess the effectiveness of interventions upon processes of aging. The presumption that interventions can alter aging processes is ancient and highly appealing. Agreement as to what sort of interventions might be effective is far more difficult to come by. The lack of agreement is the result of two major problems. First, there is far from universal agreement on the nature of aging processes or understanding of the basic mechanisms involved and therefore little consensus as to what types of intervention might be effective. Second, there is currently no scientifically validated method to test potential effectiveness of interventions.

If aging is in fact the cumulative result of more basic biological processes, then a single intervention could potentially alter the rate of aging of a given physiological function or of the organism itself. Ideally, a change in the rate of aging of an organism should be measurable in any nonrenewable cell population of the organism or in the renewal rate of the remaining cell populations. Given this theoretical construct, a change in a rate of aging should be observable throughout the life span of an organism and, therefore, the identification of valid biological markers of aging should be possible.

In order to evaluate the effects of any intervention, particularly human interventions, a testing methodology that is both scientifically valid and achievable in significantly less than the life span of the organism is essential. It is this need for a workable test strategy which has also, in part, fostered interest in the development of biomarker research and, not insignificantly, in offering the public some protection against unfounded claims for this or that product or regimen which will retard the processes of aging. Some of the concepts presented here have, at least in part been discussed by others (Ludwig and Smoke, 1980; Ludwig, 1981; Reff and Schneider, 1982; Ingram, 1983; Sprott and Schneider, 1985).

CRITERIA FOR A BIOMARKER

A very significant consideration in developing a biomarker strategy must be the validity of the underlying assumption that there are biological parameters that are better measurements of functional or physiological age than chronological age. Aside from the in vitro situation, where one can clearly delineate differences between the chronological and biological age of cells in culture by freezing and thawing the cells, and those poikilothermic animal models where chronological time and biological processes can be independently modified, almost all other experimental protocols use chronological age as an intrinsic variable. Most of the information currently available would suggest that

TABLE 1. MAXIMUM LONGEVITIES OF SOME (CAPTIVE) MAMMALIAN SPECIES

			Age	
Order	*Genus species*	*Common name*	*years*	*months*
Monotremata	*Tachyglossus aculeatus*	Australian echidna	49	5
	Ornithorhynchus anatinus	Platypus	17	0
Marsupialia	*Philander opossum*	Grey four-eyed opossum	2	10
	Didelphis m. virginiana	Northern opossum	4	10
	Dasyuroides byrnei	Kowari	6	4
	Sarcophilus harrisi	Tasmanian devil	8	2
	Myrmecobius fasciatus	Numbat	5	3
	Echymipera rufescens	Rufous spiny bandicoot	2	9
	Trichosurus vulpecula	Grey brush-tailed possum	14	8
	Phascolarctos c. cinereus	New South Wales koala	17	0
	Lasiorhinus latifrons	Hairy-nosed wombat	24	6
	Wallabia r. frutica	Bennett's wallaby	15	2
	Macropus r. erubescens	Euro wallaroo	19	7
	Dendrolagus matschiei	Matschie's tree kangaroo	15	7
Insectivora	*Solenodon paradoxus*	Hispaniolan solenodon	8	11
	Setifer setosus	Hedgehog tenrec	10	6
	Echinosorex gymnurus	Moonrat	4	6
	Erinaceus c. roumanicus	Rumanian hedgehog	7	0
	Nasilio brachyrhynchus	Short-nosed elephant shrew	4	2
	Sorex araneus	European shrew	0	3
	Scalopus aquaticus	American mole	1	11
Chiroptera	*Pteropus giganteus*	Indian fruit bat	31	5
	Noctilio l. rufipes	Fisherman bat	11	6
	Antrozous p. pacificus	Pallid bat	4	9
Primates	*Tupaia glis*	Common tree shrew	12	5
	Lemur fulvus x macaco	Brown x black hybrid lemur	39	0
	Lepilemur mustelinus	Sportive lemur	8	7
	Propithecus v. coquerali	Coqueral's sifaka	18	2
	Nycticebus coucang	Slow loris	13	4
	Tarsius s. carbonarius	Mindanao tarsier	13	5
	Cebus capucinus	White-faced capuchin	46	11
	Saimiri sciureus	Common squirrel monkey	15	3
	Ateles g. pan	Schlegel's spider monkey	33	0
	Macaca fascicularis	Crab-eating macaque	37	1
	Cercocebus a. albigena	Grey-cheeked mangabey	32	8
	Papio sphinx	Mandrill	31	8
	Presbytis pileatus	Capped langur	23	8
	Pongo p. abelii	Sumatran orangutan	59	0
	Chimpansee troglodytes	Chimpanzee	53	0
	Gorilla g. gorilla	Lowland gorilla	47	11
Edentata	*Myrmecophaga taridactyla*	Giant anteater	25	10
	Choloepus hoffmanni	Hoffmann's two-toed sloth	32	1
	Euphractus sexcinctus	Six-banded armadillo	18	10
Lagomorpha	*Romerolagus diazi*	Volcano rabbit	2	3
	Lepus europaeus	European hare	7	5

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TABLE 1. (Continued)

Order	Genus species	Common name	Age	
			years	months
Rodentia	*Aplodontia rufa*	Sewellel	3	6
	Sciurus carolinensis	Eastern grey squirrel	23	6
	Marmota marmota	European marmot	12	0
	Tamias striatus	Eastern chipmunk	7	0
	Geomys bursarius	Plains pocket gopher	7	2
	Dipodomys o. richardsoni	Richardson's kangaroo rat	9	10
	Castor canadensis	American beaver	15	10
	Pedetes c. surdaster	East African springhare	13	10
	Nyctomys sumichrasti	Sumichrast's vesper mouse	5	2
	Onychomys torridus	Northern grasshopper mouse	4	7
	Neotoma albigula	White-throated wood rat	7	8
	Lemmus lemmus	Norway lemming	0	8
	Ondatra zibethicus	Muskrat	5	6
	Tatera indica	Indian gerbil	7	0
	Psammomys obesus	Fat sand rat	2	11
	Cannomys badius	Bay bamboo rat	3	3
	Apodemus sylvaticus	Common field mouse	4	5
	Notomys alexis	Brown hopping mouse	5	2
	Cricetomys gambianus	Giant pouched rat	7	10
	Phloemys cumingi	Slender-tailed cloud rat	13	7
	Myoxus glis	Fat dormouse	8	8
	Graphiurus murinus	African bushy-tailed dormouse	5	9
	Zapus hudsonius	American meadow jumping mouse	5	0
	Allactaga euphratica	Euphrates jerboa	4	2
	Hystrix b. longicauda	Sumatran porcupine	27	3
	Erethizon dorsatum	Canadian porcupine	8	5
	Dolichotis patagona	Patagonian cavy or mara	11	10
	Hydrochoerus hydrochoaeris	Capybara	11	11
	Dinomys branicki	Pacarana	9	5
	Dasyprocta aguti	Golden-rumped agouti	17	9
	Chinchilla laniger	Chinchilla	11	4
	Capromys pilorides	Cuban hutia	11	4
	Ctenomys talarum	Tuco tuco	2	2
	Abrocoma bennetti	Bennett's chinchilla rat	2	4
	Proechimys semispinosus	Spiny rat	4	10
	Thryonomys swinderianus	Cane rat	4	4
Carnivora	*Canis latrans*	Coyote	21	10
	Urocyon cineroargenteus	Grey fox	13	8
	Tremarctos ornatus	Spectacled bear	36	5
	Thalarctos maritimus	Polar bear	34	7
	Ailurus fulgens	Lesser panda	13	5
	Mustela sibirica	Siberian weasel	8	10
	Gulo g. luscus	American wolverine	17	4
	Taxidea taxus	American badger	26	0
	Lutra canadensis	Canadian otter	21	0
	Civettictis civetta	African civet	28	0

(Continued on next page)

TABLE 1. (Continued)

Order	Genus species	Common name	Age	
			years	months
	Herpestes urva	Crab-eating mongoose	13	4
	Bdeogale nigripes	Black-footed mongoose	15	2
	Crocuta c. habessynica	Abyssinian spotted hyena	41	1
	Lynx rufus	Bobcat	32	4
	Leo t. tigris	Bengal tiger	26	3
	Uncia uncia	Snow leopard	15	4
Pinnipedia	*Zalophus californianus*	California sea lion	28	0
	Phoca v. richardi	Pacific harbor seal	32	0
	Halichoerus grypus	Grey seal	41	0
Proboscidea	*Elephas maximus*	Asiatic elephant	69	0
Perissodactyla	*Equus h. hemippus*	Syrian wild ass	35	10
	Tapirus terrestris	South American tapir	35	0
	Rhinoceros unicornis	Indian rhinoceros	40	4
Artiodactyla	*Sus scrofa*	European wild hog	21	0
	Tayassu tajacu	Collared peccary	24	7
	Hippopotamus amphibius	River hippopotamus	54	4
	Lama guanicoe	Guanaco	28	4
	Cervus e. scoticus	Scottish red deer	26	8
	Alces a. alces	European moose	17	11
	Rangifer tarandus	Reindeer	20	2
	Giraffa c. capensis	Cape giraffe	36	2
	Antilocapra americana	Pronghorn	11	10
	Taurotragus oryx	Eland	18	8
	Syncerus caffer	American buffalo	29	6
	Bison bison	American bison	26	0
	Hippotragus niger	Sable antelope	19	9
	Connochaetes t. albojubatus	Eastern white-bearded gnu	21	5
	Gazella dorcas	Dorcas gazelle	17	1

chronological age by itself is as good a predictor of functional or physiological age as any other physical or physiological parameter one can measure. Costa and McCrae (1980) have shown that any number of physical, physiological, or biochemical parameters either alone or in a multivariate composite are no better a predictor of functional age than chronological age in the human species. Do these findings mean that research on biomarkers of aging is invariably doomed to fail? Not in view of the fact that no systematic research design has to date been undertaken to assess putative biomarkers of aging in a species where there is a well-defined genetic background, with a longitudinal and cross-sectional protocol, and with extensive pathological examinations. Further, it is obvious that even among members of our own species, there are fit and active individuals well into their eighth and even ninth decades of life, while others, even in the absence of detectable disease, have limited functional capacity decades earlier. Similarly, between closely related species there are major differences in life span and comparative physiological ages under nearly identical environmental conditions. These differences must reflect parameters other than just the passage of time.

The first area that needs to be addressed is what constitutes a valid biomarker of aging. Intrinsic to this basic question are a number of proposed criteria and implicit theoretical considerations which need to be explored. We feel that biomarkers of aging processes should, at a minimum, fulfill the following criteria:

1. The rate of change of a biomarker must, at least in mathematical terms, reflect some measurable parameter which can be predicted at a later chronological age.
2. The biomarker should reflect some basic biological process of aging and certainly not the predisposition toward a disease state or some inborn error in metabolism.
3. The biomarker should have high reproducibility in cross-species comparisons of functional or physiological age versus chronological age, particularly within the same classes and certainly within the same families of species.
4. Biomarkers should change independently with the passage of time and reflect physiologic (functional) age.
5. Assessment of biomarkers should be nonlethal in animal systems and should cause minimal trauma in humans. The availability of nonlethal testing in animal model systems would permit longitudinal analyses.
6. The biomarker should be reproducible and measurable during a relatively short time interval compared to the life span of the animal.

DISCUSSION

Parameters of a putative biomarker

To elaborate on the criteria, if the parameter under study is a true biomarker of aging, the rate of change must, at least in mathematical terms, reflect some prospective predictive power. The magnitude of the change is not important, but it must be accurately measured in a short enough time period to be of predictive value. Indeed, one might speculate that a number of putative biomarkers may well reflect subtle changes in biochemical, physiological, or molecular events, particularly those which manifest themselves early in adult life or even during developmental stages. For example, even small changes in systems which directly influence regulatory or homeostatic mechanisms may more closely approximate a valid biomarker of physiological age than such well-documented large changes as those observed in maximum breathing capacity in humans after the age of thirty. This is not to imply that the latter cannot be a valid biomarker of human physiological age. Rather, the changes observed are likely to be a gross manifestation of basic underlying molecular and biochemical events, such as altered rates of synthesis in matrix proteins (collagen and elastin) and/or cross-linkages within the composite tissues of the lung system. Both of these molecular–biochemical alterations have been described in various systems in a number of species with advancing age.

Other examples of where more subtle changes might better reflect physiological status of the organism could potentially be garnered from studies designed to detect minute alterations in the maintenance of ionic or osmotic regulatory systems. The logic behind these suggestions is that only slight fluctuations can be tolerated in these systems and still be compatible with life. Such highly integrated homeostatic systems must, in fact, be tightly regulated in order to sustain life and therefore may show little change with

advancing age, even when the system is challenged. However, their potential value in biomarker research should not be overlooked. For example, a small change in a homeostatic system such as maintenance of blood pH, appropriately weighted, could have high significance in a multivariate analysis with other reputed biomarkers of aging. It may well be that there are parameters that show little or no change with advancing age, such as resting heart rate in humans, or those that demonstrate a highly constant rate of change, such as denaturation of tail collagen of mice which are in themselves not good predictors for longevity (see Harrison and Archer, p. 309 this issue). Both of these types of change, however, may prove to be most valuable in biomarker research, if only as baseline parameters against which other putative markers can be assessed. The systems presented here are, of course, only limited examples.

To reiteriate, the concept behind biomarker research is that at some point(s) in the life span of an individual, scientifically valid measurements are possible, which can better predict subsequent physiological capability than can chronological age. In this context, there are several distinct topics in biomarker research that bear further elaboration. They are as follows: a) magnitude and function of change of a biomarker; b) measurement of a putative biomarker; c) significance of variation in biomarker research; d) implications of species evolution and life history strategies for biomarker research; and e) the role of biomarker research in interventions.

Magnitude and function of change of a biomarker

As stated earlier, if the parameter under study is a valid biomarker of aging, then it must be possible to express the rate of change in mathematical terms in order to predict physiological capability at some later chronological age. The magnitude of the rate of change is not necessarily important. The only essential criterion for a change in a putative biomarker is that it can be accurately and reproducibly determined within a time frame to be of reasonable prospective value. As a general rule, it is not likely that a variable which shows measurable change only after the 50% survivorship of an experimental population would be a good candidate as a biomarker. Certainly, a change in a variable that occurs only after 70% survivorship is highly suspect as a potential biomarker. Ideally, the most useful biomarkers would be those which could be assessed very early in the life span and have predictive values later in the life span. At the molecular–biochemical level there may in fact be valid biomarkers of aging that can be determined early in life; however, it is likely that developmental and maturational processes may mask or lead to misinterpretation of a number of such putative biomarkers.

Measurable decrements in most major human physiological systems are not observed until after the age of thirty and exhibit the greatest declines only later in life (Shock, 1981). A similar situation is observed in most rodents, where most alterations in functional parameters are not observed until after 12 months of age (National Research Council, 1981).

The concept of reasonable prospective value may indeed prove to be a major difficulty for some types of biomarker research. For it is likely that a number of biomarker candidates which have been suggested as potential markers of aging may, in fact, be relatively poor predictors of physiological age and excellent predictors of impending death. A good example used earlier is maximum breathing capacity, which in humans proves to be an excellent correlate of limited life expectancy (Beaty *et al.*, 1982).

Death, although an unavoidable experimental caveat in all gerontological research, is

the least important variable in biomarker research. The *cause* of death of an individual is of importance in gerontological research in general and particularly in biomarker research. This is because in biomarker research there must be at least an implicit acknowledgement that aging is not a disease nor a cause of death. In other words, if a given experimental protocol is truly biomarker research, the parameter under investigation must demonstrate change(s) intrinsic to the processes of aging within that species. The alterations cannot be due to any extrinsic perturbations, including disease. For example, an individual may demonstrate an array of physiological capabilities which would predict above-average functional status at a later chronological age; however, that same individual may also have a brain tumor which will cause death in a matter of weeks. Although this is an overt pathology which might be easily detected, there are unquestionably a number of others, particularly in nonprimate species, that could easily confound biomarker research.

Many putative biomarkers could be better or more valid indicators of susceptibility to disease states rather than basic biological processes of aging. This particularly caveat should not, however, be viewed negatively in terms of pursuing biomarker research, but rather as an opportunity to further disentangle processes of aging from the etiology of disease. This would be particularly plausible if studies on biomarkers were carried out on animals with well-defined genetic backgrounds and well-described pathologies. This methodological approach would obviously greatly facilitate our knowledge concerning the interrelationships between fundamental aging processes and age-associated susceptibility to certain disease states.

The direction or function of a change in a putative biomarker is of no importance to its utility as a biomarker. The only criteria are that it can be accurately determined and can be used in a predictive manner as discussed earlier. The parameter under study may increase and/or decrease. It could have a parabolic, stepwise, or any other mathematical expression. We are more accustomed to observing declines in physiological and biochemical parameters and increases in such parameters as cross-linkages and lipofuscin; however, there may be certain parameters which exhibit other types of change or periodicity in change which may prove to be valid biomarkers of aging.

Measurement of a putative biomarker

Probably the most serious potential flaw in biomarker research will be in the determination of what constitutes an optimum for a given parameter. Against what baseline should a putative biomarker be assessed, because the basic premise of biomarker research is that there is some better measure of physiological age (functional capacity) than chronological age. This question becomes even more complex given the great variability of most parameters of functional capacity even within rather homogeneous populations. For example, what are the optimal standards for cognitive function, physical strength, physical dexterity, etc.? In a given species, what are the genetic optima for biological parameters? How much plasticity is tolerated? What pleiotropic effects are involved? This dilemma is exacerbated when the relationship of functional age and longevity are considered without full recognition of the potentially spurious impact of environmental perturbations, including disease as discussed above. Again, the basic criteria of a valid biomarker are that it will directly relate to functional age better than chronologial age and will in some manner predict longevity. Certainly all optima (maximal and minimal) cannot in themselves be of a high predictive value of physiological

capacity at some later chronological age. Physical strength would be a poor predictor of the functional capacity or longevity of an organism at some later time as would cardiac output, vital capacity, etc. It is apparent from this vantage point that many putative biomarkers will need to be assessed in relation to other physiological, biochemical, and/or molecular–genetic systems in order to have any validity. It is also quite evident that there may be many processes of aging and that various systems (physiological, biochemical, and molecular–genetic) undergo alterations at differential rates. The inter- and intrarelationships between various systems and the impact of these relationships on the rate of change in one system versus another must also be determined. It is readily apparent that while alterations in some systems may have widespread trophic effects, others may only have limited impact on the overall functional capacity (vitality and viability) of the organism. The use of various mathematical models to gain insight into these various relationships may be of indispensable value, not only with regard to the assessment of a given biomarker, but also to further our understanding of the basic biological processes of aging.

Significance of variation in biomarker research

If a parameter under study is a valid biomarker of aging, it must demonstrate variation of sufficient magnitude in short-term longitudinal or in cross-sectional studies to be of predictive value within a population or cohort with regard to physiological capacity at a later chronological age. Obviously, without variation any parameter would be useless as a biomarker. While variation of a given parameter is essential to biomarker research, there may be some tendency to over emphasize those parameters which demonstrate the greatest amount of variation, particularly late in the life span. This tendency is not surprising, as most biochemical and physiological parameters show increased variation with advancing age.

Increased variance with advancing age is particularly evident in studies of human physiological performance. There are exceptions even in human systems, which need to be evaluated. For example, lens accommodation capacity in human cross-sectional studies is optimal between the ages of 5 and 8 years at 13 diopters with a large variation and decreases in an almost linear fashion until ages 50 and 52 to about 1.5 diopters with small variation (Carter, 1982). The effects of the passage of time on some biological processes are self-evident, although not necessarily absolutely fixed, and certainly more variable in some stages of the lifespan than in others. For example, embryogenesis, gestational periods, developmental and maturational processes are generally more accurately timed at earlier stages and show increasing variation at later stages in most animal species. However, they may not necessarily reflect relative biological time.

Figure 1 illustrates this point for developmental and various life stages as well as for physiological and biochemical parameters (Fig. 2) in *Drosophila melanogaster*.

While it is clear that there is an increase in the variation of all the parameters presented in Figs. 1 and 2, the interdependency of chronological time and biological processes must not be overlooked in assessing the relevance of variation in a parameter with time. For example, if one examines the relative time variation in each of the life stages of *Drosophila* in terms of actual time to complete a life stage process, there is less absolute variation in earlier stages of the life span than is observed in successive stages. While the differences between chronological time and the rates of biological processes

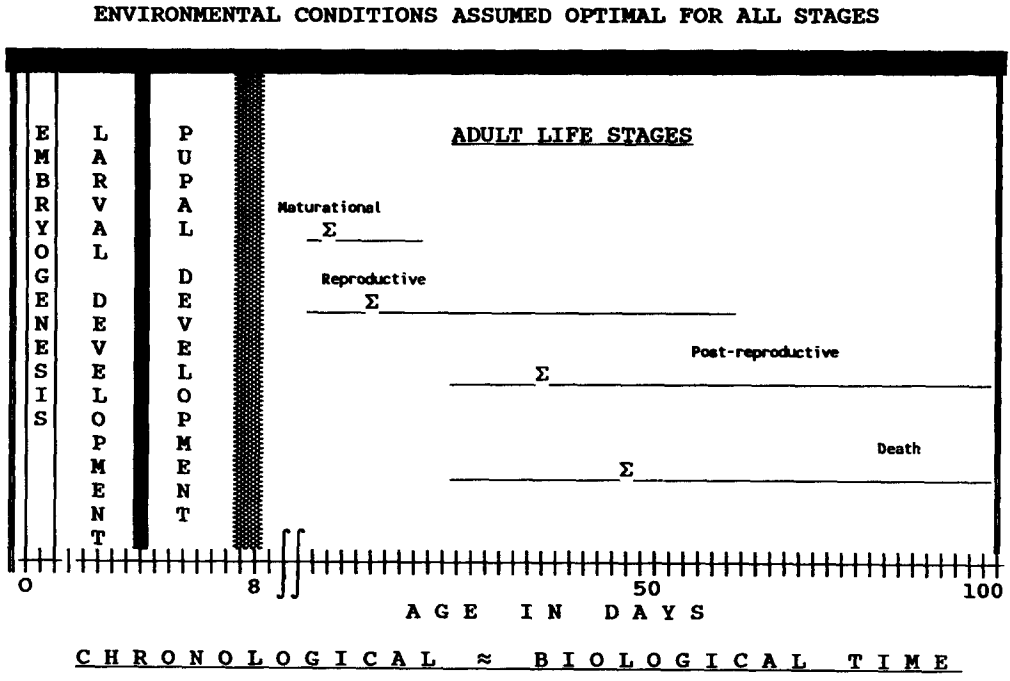

FIG. 1. The absolute time and variation of the life stages for *Drosophila melanogaster* (Sevelan strain) under rigidly controlled environmental conditions. The vertical lines represent the mean and SD (width of line) for developmental stages. The actual numbers are: embryogenesis, 18 ± 0.21 h; onset puparium formation, 141.6 ± 4.8 h; and eclosion, 206.4 ± 9.6 h. The calculated relative time variations for each stage (SD ÷ Mean) are 0.012; 0.034; and 0.047, respectively, for embryogenesis, puparium formation, and eclosion. Viability for egg to puparium ratio was 75% and 99% for puparium to imago. These data are based on 606 animals reared 30 per 80 ml vial under conditions previously described (Baker, 1978). The adult stages for females are presented as the mean and range of maturational time (mean represents onset of viable egg laying capability); length of reproductive life span (mean represents optimal egg laying capability); postreproductive life span (less than two viable eggs/day), and age at death. These data are taken from various experimental protocols on fly populations (Sevelen strain) of generally more than 1000 animals and therefore not necessarily directly comparable. (Unpublished data as presented.)

are the essence of biomarker research, the two phenomena must co-exist in the same spatial frame and therefore, in any experimental design they are interdependent. It is the variation in the expression of biological processes in a given temporal frame within individuals of a population that undoubtedly will provide some of the best biomarkers of aging processes, rather than the magnitude of change or the variation in change with time. In other words, the absolute value of a given biological parameter is less likely to be of importance in biomarker research than the temporal pattern of change in that parameter. For example, in individuals with equal life spans, one individual might have a relatively low cardiac output with late onset of change while in another individual the onset of change might occur earlier in the life span but proceed at a significantly slower rate.

Figure 3 illustrates the potential differences in absolute values, onset of change, and rate of change of a putative biomarker of aging processes and the predictive value of that

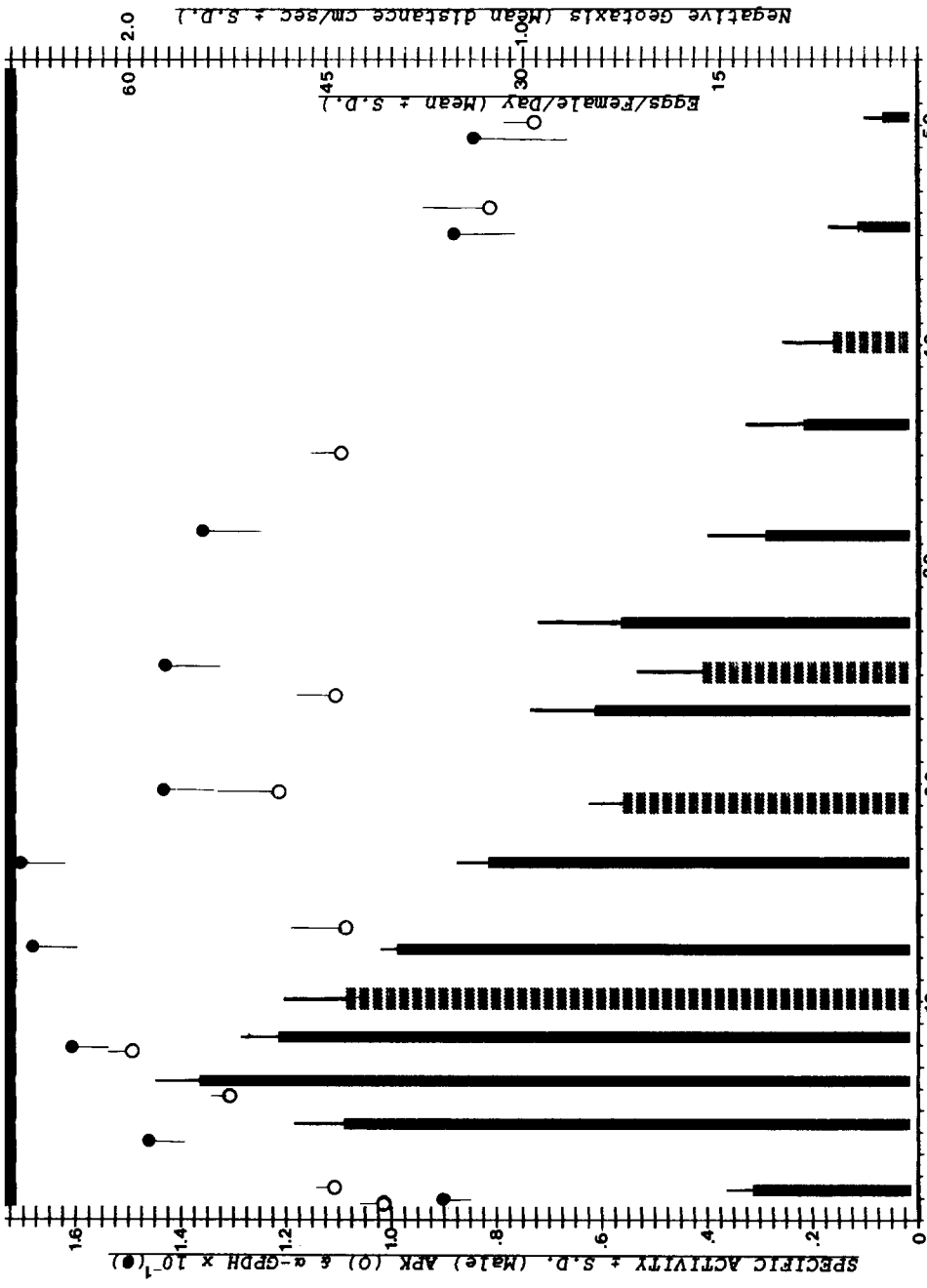

FIG. 2. The solid bar represents periodic fecundity ± SD and the broken bar presents locomotor ability as determined by timed negative geotaxic response ± SD. The open circle shows the specific activity of L-arginine phosphotransferase ±SD (APK) in males with advancing age and the closed circles the activity of α-glycerophosphate dehydrogenase ± SD (α-GPDH), also in males. The fecundity and negative geotaxic data are unpublished whereas the enzyme data is adapted from Baker (1975), Baker *et al.* (1978), and Baker *et al.* (1985).

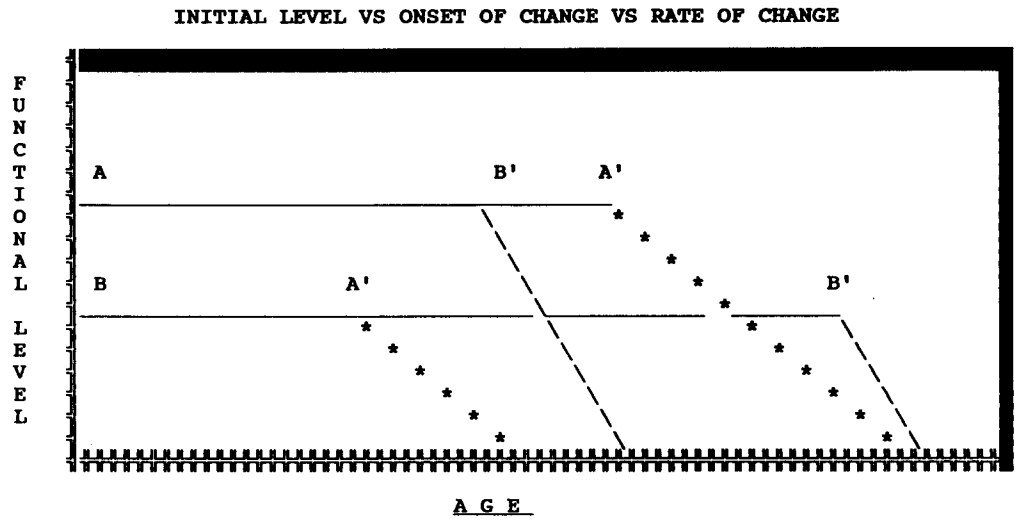

FIG. 3. Illustrates empirically the potential differences that may occur in individuals for a given putative biomarker where A and B represent two different initial functional levels, A′ and B′ represent different time of onset of change, and the dashed and broken lines represent different rates of change for both A, A′, B, and B′.

parameter. As is shown where A and B represent initial values of a biological parameter and A′ and B′ represent those same values but exhibit a later onset in the decline, it is the rate of decline which can ultimately be more critical than the initial value or the time of onset of that change.

Implications of species evolution and life history strategies for biomarker research

If a biological parameter were a valid biomarker of aging, one would expect it to have a high predictive value in cross-species comparisons, at least within the same phylogenic family, and to a lesser extent within the same order and class, respectively. Cross-species validation of biomarkers is an important consideration in biomarker research, as many studies which will eventually be extrapolated to humans will first be done in laboratory animals. It is in this context that an understanding of the evolution and life history strategies of a species becomes relevant. Life span is an evolved coadapted trait of a life history strategy for a given species. For evolution there is only one criterion of success; the survival of the species. For a given ecological niche, a species will evolve through selection pressures to fit the ecologically defined parameters of that niche. This will entail the development of those molecular–genetic, biochemical, physiological, and anatomic attributes and life history strategies within a species that best facilitate the exploitation of that niche. It will be those biological characteristics which best ensure the survival of the species which will be developed, namely, those which optimize reproductive success. The diagram shown in Fig. 4 illustrates empirically how any physiological or biochemical system would be under selection pressure to optimize its development in concert with the optimization of reproductive capacity for a given ecological niche.

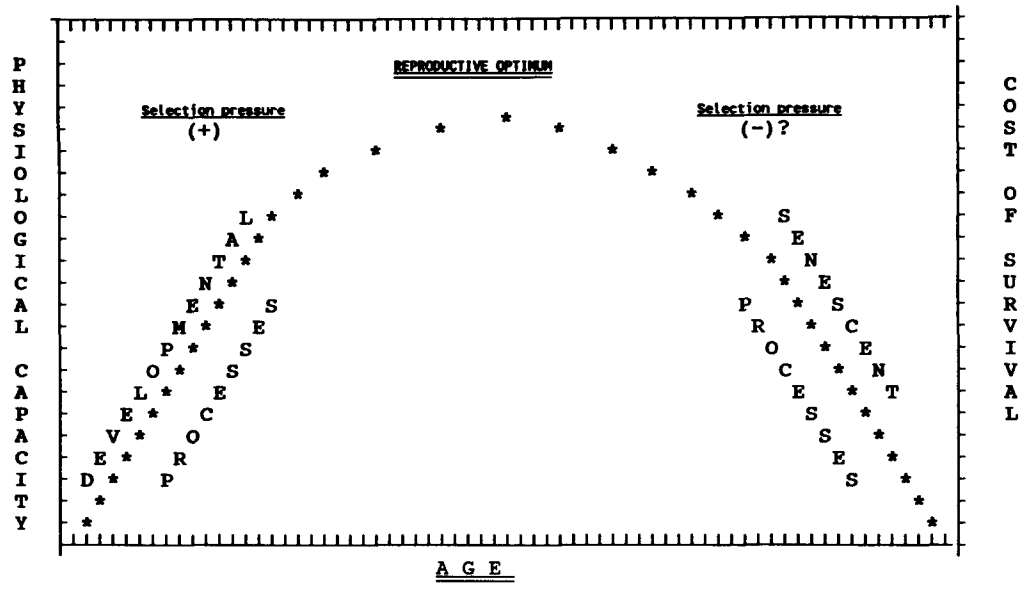

FIG. 4. An idealized schematic of how selection pressure might be operationally manifest in optimizing reproductive capacity for a species and at least in a neutral manner influence the rate of aging for that species (see text for further discussion).

It is not incidental that those very evolutionary mechanisms that optimize reproductive capacity in a given species are also those determinants which will directly or indirectly influence the maximal life span of that species. The only selection pressures operative in the postreproductive period of an individual's life span would act on those traits which somehow enhance survival of the progeny. These traits could enhance species specific longevity and might include prolonged physiological vigor in some systems to ensure successful rearing of progeny to reproductive age (Christian and Baker, 1979). The best example of such positive selection might be that for knowledge (information) transfer in humans. Indeed, cognitive functional capacities are remarkably well-preserved in comparison to other physiological systems in humans (Shock *et al.*, 1984) and the evolutionary worth of older individuals may outweigh the lack of a direct reproductive contribution by enhancing the overall fitness of the population through knowledge transfer. There is, in fact, paleontological evidence from our own immediate evolutionary history that older, even infirm, individuals were maintained in pre-*Homo sapien* communities in spite of the fact that they presumably utilized resources which might be better used by younger cohorts to enhance survival of the species. Similarly, in other species which exhibit postreproductive life spans, such as the elephant, there are specific roles for older individuals within a societal structure that enhance the survival of progeny.

In cross-species analysis of putative biomarkers, lack of a correlation of a particular parameter in one species versus another may reflect different evolutionary priorities in optimization of a specific system. For example, the visual systems of predatory birds are highly developed, whereas those of elephants, a much longer-lived species than most predatory birds, is relatively less well developed. Similarly, the visual systems of some

cephalopods is highly developed and yet they have comparatively short life spans. Each of these apparent discrepancies, however, has clear survival advantages for each species. An alteration in one system of a given organism need not necessarily be mirrored exactly in another, even relatively closely related, species to have validity as a biomarker if there can be demonstrated that a particular trait imparts a survival advantage to that species. Among closely related species, however, there should more often than not be a high correlation of a putative biomarker.

Just as biological systems have evolved within species to best exploit an ecological niche, so have life history strategies evolved. These strategies can dramatically affect the mean life span of an organism and invariably are linked to reproduction. For example, Pacific salmon have a life expectancy of three years on average. However, if a salmon is prevented from swimming upstream to spawn, life expectancy can be extended threefold. A similar scenario can be written for a number of semilparous species, such as the octopus, which have one massive brood then undergo a rapid senescence and death (Wodinsky, 1977). There are a number of other known and undoubtedly yet to be described examples of modifiable programmed death linked directly or indirectly with reproduction, primarily through underlying hormonal mechanisms. Although this type of life history strategy is most often found in lower animal species, it has also been observed in the Australian hopping mouse, where the level of epinephrine rises so rapidly after copulation in the male it causes a cerebrovascular accident. An individual of a given species may, through the evolution of a specific life history strategy, be set on a course of predictable cause of death not related to the basic biological processes of aging for that species. This is not to say that biomarkers cannot have validity in such species, only that the molecular–genetic, biochemical, or physiological parameters assessed may predict physiological age but not longevity, if the life history strategies are not fully understood.

The role of biomarker research in interventions

An assessment of biomarkers would be incomplete without discussion of possible interventions. In general, three classifications of interventions may be described: a) those which extend maximum lifespan; b) those which increase the mean lifespan (rectangularization of the mortality curve); and c) those which have segmental effects, that is, those which affect a physiological or other system such as bone loss, cardiovascular performance, or immune function. The latter type of intervention may or may not be reflected in either of the former types of intervention. As more evidence accummulates to suggest that there are multiple mechanisms involved in the aging of an organism, the possibility of effective segmental interventions becomes plausible and potentially highly rewarding. This is not meant to suggest that the potential interventive methodologies derived from biomarker research should in any way supplement or detract from what should be the main thrust of all serious biogerontological research, namely, the understanding of the underlying basic biological mechanisms ultimately responsible for the processes of aging.

The best documented single intervention which has been shown to effect all three of the aforementioned classifications of interventions is caloric restriction in laboratory rodents (see Masoro, p. 391 this issue). This intervention has been demonstrated to alter mean and maximum longevity and to have beneficial segmental effects. The mechanism(s) of caloric restriction effects is poorly understood. Indeed, there is some ques-

tion that the effect may be an artifact. A caged *ad libitum* fed rodent has generally been used as the control group in these studies. However, dietary restriction does alter the slope of the Gompertz curve in calorically restricted rodents and thus may, in fact, alter the rate of aging rather than merely act to prevent certain diseases occurring (Sacher, 1977).

There are a number of other proposed types of interventions (Schneider and Reed, 1985). These range from administration of various antioxidant compounds, vitamins, amino acids, pharmacologically active substances, and hormones, to various diet and exercise regimens. The efficaciousness of such interventions, some potentially highly deleterious, has not been proven, in part because there exist no reliable, scientifically valid standards against which to assess these interventions. The development of biomarker research should greatly enhance our abilties to assess various types of interventions, particularly at the segmental level.

There is still a relative paucity of information regarding the more basic molecular–genetic mechanisms of aging processes and many may argue that, given this situation, it is ill-advised to direct efforts towards the development of assessment tools (biomarkers). However, if the research on biological markers of aging is appropriately designed, there is no reason to believe that significant insights into the more basic mechanisms of aging will not be forthcoming. This would be particularly true if putative interventions are experimentally used as probes to understand more basic mechanistic processes.

NATIONAL INSTITUTE ON AGING INITIATIVE

If biomarker research is ultimately to be successful, it is clear that biomarkers in species other than the laboratory rodent must be developed. While there are great similarities between processes of aging in rodents and humans, there are many examples of marked differences in functions. The ideal animal model should be phylogenically closer to humans. Another primate species would be most useful in this regard. The National Institute on Aging does support a colony of pig-tailed mascques *(Macaca nemistrina)* for biomarker research. The cost of these animals and the requirement of nonlethal and minimally traumatic procedures, however, may limit their usefulness in the development of biomarker research. The laboratory rodent will, therefore, continue to contribute to the study of biomarkers and interventions. The extensive characterization of some strains of rats and mice, as well as their relatively short life spans make them the animal models of choice for development of biomarker research at this time.

In order to provide the opportunity for biomarkers of aging development under optimum conditions, the National Institutes on Aging has begun a ten-year initiative. The basic assumptions of this initiative are a) that biomarker development has been hampered historically by inadequate resources, heterogenious resources, procedures, and environments and by a lack of clear-cut, generally understood objectives; and b) that the provision of a carefully chosen array of genetically defined animal models raised on well-defined diets in a controlled environment will enhance the comparison of results across laboratories.

The core of the National Institute on Aging initiative is a colony of animals being developed in collaboration with the National Center for Toxicology Research. The colony consists of seven rodent genotypes (F344, Brown–Norway and F344 × BN F1

hybrid rats; C57BL/6NNIA, DBA/2NNIA, B6D2F1NNIA, and C3B6F1NCRT mice) ranging in age from three to thirty months. Animals of each genotype are being maintained *ad libitum* and on calorically restricted diets, and a portion of the animals in every genotype, sex, diet, and age cohort are being set aside for cross-sectional pathological characterization and for life span morbidity and mortality studies. Animals from this colony will be provided to investigators who successfully compete for biomarker grants to be awarded for this purpose. The National Institute on Aging expects to award eight to ten such grants in early 1988.

SUMMARY

This article has examined some theoretical and conceptual aspects of biomarker research which are solely the thoughts of the authors. A description of the National Institute on Aging initiative in this area is also included. The two components should not be taken as synonymous. As the development of biomarker research is a relatively new concept, there will most certainly be a rapid infusion of fresh thought into the concepts set forth here. This presentation is only one viewpoint in biomarker research, which hopefully will be critiqued, challenged, modified, and enhanced. We are expectant, however, that the initiative taken by the National Institute on Aging in establishing the rodent colonies for specific use in biomarker research will have a significant impact on our understanding of rates of change in various biological parameters with advancing age. The inter- and intrarelationships between different systems and/or biological parameters on aging of the whole organism should become clearer as biomarker research progresses. And, hopefully with appropriately designed experimental protocols, there will be a further understanding of the interrelationships and perhaps the intrarelationships between processes of aging and the processes of disease as a result of the data this initiative should generate.

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REFERENCES

BAKER, G.T. Identical age-dependent patterns of enzyme changes in dipteran insects. *Exp. Gerontol.* **10,** 231, 1975.

BAKER, G.T., III. Age-dependent alpha-glycerophosphate dehydrogenase activity changes in wild type, vestigial winged and alpha-glycerophosphate dehydrogenase deficient mutants of *Drosophila*. *Mech. Ageing Dev.* **7,** 367–374, 1978.

BAKER, G.T., III, AQUET, N.J., PELLI, D.A., CROSSLEY, K.L., and ROSSNICK, J.B. Mechanisms of age-related enzyme activity changes in *Drosophila melanogaster*. *Fed. Proc.* **37,** 880, 1978.

BAKER, G.T., III, JACOBSON, M., and MOKRYNSKI, G. Aging in drosophila. In: *Handbook of Cell Biology of Aging*. Cristofalo, V.J. (Editor), pp. 511–578, CRC Press, Inc., Boca Raton, FL, 1985.

BEATY, T.H., COHEN, B.H., NEWILL, C.A., MENKES, H.A., DIAMOND, E.L., and CHEN, C.J. Impaired pulmonary function as a risk factor for mortality. *Am. J. Epidemiol.* **116,** 102, 1982.

CARTER, J.H. The effects of aging on selected visual functions: Color vision, glare sensitivity, field of vision, and accommodation. In: *Modern Aging Research* Vol. 2., *Aging and Human Visual Function* Sekuler, R., Kline, D., and Dismukes, K. (Editors), pp. 121–130, Alan R. Liss, Inc., NY., 1982.

CHRISTIAN, R.R. and BAKER, G.T. Influence of postreproductive cohorts in the selection for increased longevity. *Gerontologist* **19**, 61, 1979.

COSTA, P.T. and McCRAE, R.R. Functional age: A conceptual and empirical critique. In: *Proceedings of Second Conference on Epidemiology of Aging*, Haynes, S.G. and Feinleib, M.F. (Editors), pp. 23–50, NIH Publication No. 80-969, 1980.

HARRISON, D.E. and ARCHER, J.R. Biomarkers of aging: Tissue markers future research needs, strategies, directions and priorities. *Exp. Gerontol.* **23**, 309–321, 1988.

INGRAM, D.K. Towards the behavioral assessment of biological aging in the laboratory mouse: concepts, terminology and objectives. *Exp. Aging Res.* **9**, 225–238, 1983.

LUDWIG, F.C. and SMOKE, M.E. The measurement of biological age. *Exp. Aging Res.* **6**, 497–522, 1980.

LUDWIG, F.C. Senescence: Pathology facing medicine's ultimate issue. *Arch. Pathol. Lab. Med.* **105**, 445–451, 1981.

MASORO, E.J. Physiological system markers of aging. *Exp. Gerontol.* **23**, 000–000, 1988.

MAYER, P.J. and BAKER, G.T., III. Developmental time and adult longevity in two strains of *Drosophila melanogaster* in a minimal stress environment. *Mech. Ageing Dev.*, **26**, 283–298, 1984.

NATIONAL RESEARCH COUNCIL — Committee on Animal Models for Research on Aging. Mammalian models for research on aging. National Academy Press, pp. 1–587, 1981.

REFF, M.E. and SCHNEIDER, E.L. (Editors). *Biological Markers of Aging*. NIH Publication No. 82-2221, 1982.

SACHER, G. Life table modification and life prolongation. In: *Handbook of the Biology of Aging*. Finch, C.E. and Hayflick, L. (Editors), pp. 582–638, 1977.

SCHNEIDER, E.L. and REED, J.D. Modulations of aging processes. In: *Handbook of the Biology of Aging*. Finch, C.E. and Schneider, E.L. (Editors), pp. 45–76, 1985.

SHOCK, N.W. Indices of functional age. In: *Aging: A challenge to Science and Society*. Danon, D., Shock, N.W., and Marios, M. (Editors), pp. 270–286, Oxford University Press, Oxford, 1981.

SHOCK, N.W., GREULICH, R.C., ANDRES, R., *et al. Normal Human Aging: The Baltimore Longitudinal Study of Aging*. NIH Publication No. 84-2450, 1984.

SPROTT, R.L. and SCHNEIDER, E.L. Biomarkers of aging. In: *Proceedings of the Inter. Conference on Nutrient and Aging*. Hart, S. (Editor), pp. 43–52, A.R. Liss, New York, 1985.

WODINSKY, J. Hormonal inhibition of feeding and death in octopus: Control by optic gland secretion. *Science* **198**, 984–951, 1977.