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MINIREVIEW

A TIME TO DIE

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Summary: A theory of dying is proposed. The evidence and arguments are presented which suggest that the lifespan of mammals is regulated by a biological clock which, in turn acts on the endocrines to produce failure of two specific target tissues, the immune and circulatory systems. Failure of these two systems can account for the similarity among mammals of the final diseases recorded at autopsy. A newly described pituitary factor is used as an example of a possible endocrine mechanism by which the body might control its own demise.

During the course of our investigations into pituitary-thyroid relations, a pituitary factor was discovered that, with advancing age, may decrease the response of peripheral tissues to thyroid hormones (1). This discovery led to a reconsideration of the earlier hypothesis (2) that hypothyroidism contributes to the pathophysiology which leads to death. The new endocrine findings have been integrated with a variety of other facts into the following theory of dying. Frequently, theories of aging and theories of dying are presented as if they were dealing with the same phenomenon and as if the same facts were equally relevant to death and aging. Rocks, plants and men age. "To age" means to change with time. Some changes with age are causally related to death, some are not.

Three rules have been used to select the data uniquely suited to a theory of dying for mammals. Facts are included only when they might be causally related to the diseases, described at autopsy, which are the immediate causes of death. Facts are included from experiments which foreshorten life only when the resulting death

resembles in all respects a "natural death." At present, no facts can fit this rule. Data from experiments which prolong life are included only when the lifespan of the strain has been extended approximately 50% beyond the average lifespan for the species. This rule prevents inclusion of experiments which may have extended the lifespan of an inbred laboratory animal by curing an unsuspected genetic defect.

The following are the major facts which meet the preceding rules. a) Each species of mammals has a characteristic lifespan. b) The lifespan of a particular species appears to be correlated with a variety of other major species-specific timed functions (gestation, puberty, heart rate, metabolic rate). c)Excluding accidents and certain genetic diseases, mammals appear to die from the same final diseases as determined at autopsy (3). d) A growing body of evidence suggests that the final diseases are themselves caused by the failure of only two major systems of the body, the circulatory and the immune. Failure of the circulation is acknowledged as the major cause of death in modern societies. Failure of the immune system is strongly implicated as the cause of the increasing sucseptibility with advancing age to both infection and cancer. e) There is a gradual and linear decline in many bodily functions in middle and old age, during a period of time when the rate of dying is increasing exponentially (4). f) McCay was able nearly to double the lifespan of rats by partial, chronic starvation (5). Both microscopic and macroscopic examination of these partially starved rats indicated the absence of diseases such as infection and cancer at an age when these diseases were commonly found in controls which were fed ad libitum. As McCay's experiments are the only ones that produced marked prolongation of life, any theory of dying must be considered incomplete unless it offers an explanation for his results.

The following outline of a theory of dying is based on

the preceding facts. It is suggested that four causes of death act in a programmed sequence to kill the animal. 1. There is a genetically controlled biological clock. 2. This clock acts by means of some endocrine effector system to cause the gradual deterioration of the circulatory and immune systems. 3. Deterioration of these two systems causes a decrease in the ability of animals to resist diseases. 4. These diseases are described at autopsy as the immediate causes of death. Each of the four causes of death will be discussed in the above sequence.

1. Biological clock: From the time of Rubner (6) to the present (7) various authors have noted that the ratio of the major timed events of mammals to their lifespans is nearly constant despite large differences in the time for the events. The present calculations are based on the maximal lifespan of 36 mammals (Table I).

I
TABLE

Mammals Used for Calculations and Maximal Lifespan (years)

Man	113	Sheep	20	Mink	10
Elephant	60	Seal	19	Phalanger	9
Horse	50	Bat, large brown	18	Red squirrel	8
Chimpanzee	39	Goat	18	Guinea pig	7.7
Whale	36	Armadillo, 6 band	18	Opossum	7
Dog	34	Raccoon	16	Muskrat	6.3
Cattle	30	Squirrel	15	Garden Mouse	6
Rhesus monkey	29	Artic fox	14	Kangaroo rat	5.5
Swine	27	Marmot	14	Rat	5
Bear	26	Rabbit	14	Mouse	3.3
Bat, small brown	22	Platypus	14	Hamster	2.5
Cat	21	Reindeer	12	Kangaroo mouse	1.3

Range of lifespan: 86-fold
Range of body weight: 11 million-fold
References 8-12 for this Table and Table II and III

The correlation coefficients of the ratios of lifespans plotted as a function of the four other parameters are significant (Table II).

II

TABLE

Correlation Coefficient Between
Maximal Lifespan and Bioparameters

	Puberty	Gestation	Heart Rate	Basal Metabolic Rate
r	0.82	0.66[a]	0.65 (.92)[b]	0.58 (.71)[c]
z	11.47	8.61	3.75	4.4
k	5.32	.104	37.8	1.62
n[d]	25	28	21	31
p value	.001	.001	.001	.001

r= correlation coefficient calculated by least squares; z= intercept on y axis, k= slope in formula: $x = ky + z$.

[a] Not including opossum, as it is a marsupial

[b] Not including man, little brown bat, squirrel.

[c] Not including man, little brown bat, large brown bat.

[d] Since data for all parameters were not available for all species, the number of points used for calculations were less than the total number (36) species used.

Another way of calculating the relationship between lifespan and the other parameters is to plot the distribution of the ratios of lifespan/parameter in terms of means and standard deviations (Table III).

III

TABLE

Constants for the Determination of
Lifespan in Years from Other Bioparameters

	Puberty (years)	Gestation (days)	Heart Rate (sec/beat)	BMR (sec/cc O_2/kg)
k	23.67	0.181	50.76	2.28
std. dev.	±15.67	±.113	±47.60	±1.64
k			34.47[a]	1.85[b]
std. dev.			±12.88	±.71

[a] Average for constant determined without man, little brown bat, squirrel.
[b] Average for constant determined without man, little brown bat, large brown bat.

This method of calculation assumes in the formula, $x = ky + z$ that $z = 0$. Plotting the data in this manner readily permits the detection of markedly deviant species or groups of species. It is suggested that the variability in Tables II and III might be considerably reduced if the species were grouped into mammals of generally similar types and if peculiarities related to biological specialization were taken into consideration. For example, only eight out of 105 ratios used in the calculations fell more than two standard deviations from the mean. Six of these markedly deviant ratios were attributable to man and the two species of bats. In the case of bats, it is of some interest that for 20 hours a day they effectively hibernate and become poikilotherms (13). Therefore, if their heart and metabolic rates were averaged for their lifetime, the average rates would be considerably slower than the rates measured when they were fully awake. It must be emphasized that the data and calculations in Tables I- III can only be used as a preliminary test for the hypothesis because the number of species is limited and the primary data were collected by a variety of investigators under different conditions.

While admitting the limitations of the above data, nevertheless the calculations did suggest that a common timing mechanism (biological clock) controlled the rates of the various parameters. Before presenting the model for the biological clock, a general analysis will be presented of the main components of any complex timed system. A timed mechanical or biological system requires three basic components: an oscillator, a coupling device and an effector. A stable oscillator is required to produce an analog of real time. For example, in an automated factory a synchronous motor (electric

clock motor) might be used. Since different tasks in the factory must be controlled at different rates, some coupling device, such as gears, is needed between the oscillator operating at a fixed frequency and the final effectors (switches, valves) which actually control the machinery. For example, proper gear selection assures that events A, B and C will occur every 2, 6 and 1000 minutes. If one wished to double the rate of production in the factory, one could simply double the frequency of the oscillator. Events A, B and C would occur in 1, 3 and 500 minutes but the ratios of each event to each other would remain the same (A/B =.33, A/C =.002) Since the ratio of the events to each other and their sequence would be unchanged, none of the complex coupling and effector parts of the timing system need be changed. Additionally, the machinery itself could also remain the same.

The preceding general considerations and the data in Tables II and III stimulated a search for a possible molecular mechanism which could act as the oscillator for the biological clock. In the case of the heart, it is known that the rate of ion (sodium, potassium) leakage across the membranes of pacemaker cells controls the heart rate. In addition, Whittam and Wheeler (14) have suggested that the rate of sodium and potassium leakage across cell membranes may be the "pacemaker" mechanism which controls the rate of resting cellular respiration. Since the sum of the respiratory rates of major individual tissues measured <u>in vitro</u> equals the metabolic rate of whole animals (15), it is possible that ion leak rates might control the metabolic rate as well as the heart rate. It is an intriguing possibility that ion leak rates may also control the rates of pacemaker cells in the central nervous system which are responsible for neuroendocrine control of development.

Although physical pore size cannot be used as a completely

reliable index to predict the rates of passive diffusion of ions, (16) it is of some interest that the areas of the "pores" of red cells from dogs, men and cattle form a rank order of decreasing area (17). In 13 out of 15 cases the species with the larger pores had faster rates for the five bioparameters studied in Table II. Unfortunately, comparable interspecies data is unavailable for a more typical cell than the red cell.

In summary, it is suggested that the rate of the major timed events of mammals might vary directly as a function of ion leakage and constants could then be calculated for the events as a function of leakage.

2. Couplers and effectors of the biological clock: The proposed mechanism for the oscillator of the biological clock is highly speculative. However, there is some evidence that mammals change the rate of the timed events by changing the rate of the oscillator, rather than by changing the coupler and effector systems.

The data in Table III indicate the coupling systems must be relatively similar for a wide range of mammals because the ratios among the five parameters are relatively constant (see factory analogy above).

In the case of puberty, gestation, heart and metabolic rates the endocrines appear to be the effectors and these effectors bear a striking similarity to each other among different species. For example, at puberty, thyroid hormones are required for the normal development of the pituitary/hypothalamic systems that are responsible for sexual maturation and growth. The pituitary gonadotrophic and growth hormones of different species are similar in structure. The testicular and ovarian hormones are identical among different species. Gestation appears to be controlled by similar or identical hormones (sex steroids, pituitary and placental polypeptides). Finally, heart

rate (thyroid hormones, catecholamines, acetylcholine) and the basal metabolic rate (thyroid) also appear to be controlled by the same hormones among different species.

While four out of the five timed parameters appear to be endocrine-controlled, up to the present there has been little clear evidence to suggest that death is also under endocrine control. However, recent findings in our laboratory (1) suggest there may be a developmental endocrine system which causes a progressive, potentially debilitating endocrinopathy, hypothyroidism, with advancing age.

As noted above, early in this century hypothyroidism was considered to be the most likely endocrinopathy which could contribute to the pathological processes associated with old age. (2). There were striking similarities between the clinical appearance of young persons with severe hypothyroidism and "normal" aged persons. In addition, the supposedly specific test for alterations of thyroid state of the peripheral tissues, the basal metabolic rate, declined with advancing age (11, 12). Four facts emerged to discredit this hypothesis. a) The specificity of the BMR for the thyroid hormones was questioned (18). b) Thyroid hormone replacement failed to rejuvenate older persons. c) The rate of decline in the BMR with advancing age was considered to be too slow to account for the dramatic increase in mortality rate which occurred after middle age. d) Plasma protein bound iodine levels remain constant throughout life (19). (However, more recently total tri-iodothyronine levels were found to decline slowly with age (20).)

Recent discoveries in our laboratory (1, 18, 21) tend to cast doubt on the interpretation of the facts previously used to discredit the original hypothesis. Over a number of years methods were developed to measure in the rat a new parameter, minimal O_2 consumption (18, 21). Unlike the BMR, minimal O_2 consumption appears to be alt-

ered specifically only by changes in thyroid state of the peripheral tissues among the 70 endocrine and non-endocrine factors examined (18). However, despite its apparent greater specificity, minimal O_2 consumption, like the BMR, was found to decline with age (1).

Investigations were done to determine the cause of the decline of minimal O_2 consumption with age. These experiments resulted in the discovery of a new pituitary factor (1). As judged by minimal O_2 consumption, this factor apparently emerges at puberty in the rat and its effect increases gradually throughout life (1). Since it required three times as much thyroid hormone (thyroxine (1), triiodothyronine, unpublished observations) to produce the same rise in minimal O_2 consumption of older rats compared to younger ones, it is postulated that this factor blocks the responsiveness of peripheral cells to thyroid hormones with advancing age.

Additional evidence that the response of peripheral tissues to thyroid hormones may change with age comes from the work of Hemon (22). Hemon measured the age changes in levels of alpha glycerophosphate oxidase (E.C. 1.1.99.5), an enzyme which has been reported to vary relatively specifically with changes in thyroid state of adult rats. The levels of this enzyme parallel the changes with age in the MOC in that they rise to a peak at 21 days and fall rapidly thereafter.

In view of the discovery of a factor which may decrease the responsiveness of peripheral cells to thyroid hormones, thyroid hormone replacement in elderly persons would be expected to fail and relatively constant protein bound iodine levels (19) throughout life cannot be used as an argument for physiological euthyroidism.

The discovery of such a pituitary factor also leads one to think of an endocrine explanation for McCay's experiments with partial chronic starvation. In these experiments, McCay found that partial

starvation prolonged life only when started before but not when started after puberty (5). This suggests starvation slowed the development of some endocrine program associated with puberty. It is of interest that the new pituitary factor appears at puberty and that partial starvation (1) when started before puberty but not when started after puberty retarded the normal development of this factor (1). Since it appears that this factor may be deleterious, by causing progressive hypothyroidism, the retardation of its normal rate of development by starvation might have contributed to the longevity of McCay's rats.

One criticism of this theory remains to be discussed; namely, the establishment of a causal relationship to death of any function, such as the postulated decline of thyroid hormone effectiveness, when the rate of decline with age is slow at a time when there is an exponential rise in the death rate. The slow, steady decline in such functions has discouraged some investigators from attributing the rise in death rate to so undramatic a change. However, various authors (for review 4) have demonstrated how small decreases in body functions can account for the rapid rise in death rate. The Strehler-Mildvan theory (4) is a particularly attractive solution to the problem because it includes a number of factors left out of other, similar theories. The Strehler-Mildvan theory contains a parameter V^1, which is defined as the weighted average viability index of the individual at a given age. The authors did not discuss in detail the difficult task of estimating the average decrease in the viability index from the known decrease of a single function. Clearly, there is a hierarchy of physiological functions; some functions affect V^1 to a relatively greater degree than others. The decline in some functions which, initially, are not deleterious to other systems might alter V^1 only

modestly. The decline in a function which causes a simultaneous decline in many other systems will decrease the average V^1 to a relatively greater degree. It is suggested that the decline in a major endocrine system, such as the thyroid, which affects many systems, is an example of the latter type of function.

3. and 4.) Immune and circulatory systems and disease: It has already been noted that the diseases as determined at autopsy can be largely attributed to failure of the immune and circulatory systems. There is considerable clinical evidence which suggests that the thyroid is necessary for the optimal function of these two systems. However, it remains to be shown that the new pituitary factor which may act by blocking the physiological effectiveness of thyroid hormones can cause sufficient damage to these two systems to account for their decline and the diseases which follow.

Experiments are in progress to determine how alterations in thyroid state and alterations in the levels of the pituitary factor can affect the immune and cardiovascular systems. The following are given as examples of encouraging preliminary work in our laboratory. Experiments with skin grafts (heterologous) suggest a considerable restoration of youthful immune competence is possible in older rats after the pituitary factor has been removed by hypophysectomy. Initial results suggest that relatively hyperthyroid rats (young rats, older rats given exogenous thyroid hormones) have a lower total peripheral vascular resistance, less sensitivity to the vasoconstrictor properties of norepinephrine infusion and higher O_2 consumption rate in response to norepinephrine compared to relatively hypothyroid rats (thyroidectomized young rats, normal older rats). These experiments agree with the relevant work in man (23), and are sufficiently encouraging so that the effects of the pituitary factor on vascular tone and reactivity to norepinephrine are also being investigated.

Because the above theory proposes a precisely timed and regulated lifespan for mammals one might ask: why death? Some authors feel that a program for death has selective advantages. Others disagree (for review see 24), arguing that most animals in the wild die of "accidents" before they reach "old" age and, consequently, a program for "natural" death would have no selective advantage. This argument is valid only if one postulates that the program for death does not start early in life to reduce the viability of the animal. However, the present hypothesis suggests that the program for death does start early and physiological data support the model. For example, young men, 18-20 years old, have the fastest psychomotor reaction times. If survival in the wild depends on the fastest response, either for attack or withdrawal, these young men have a distinct survival advantage over men 10-20 years older. Since hypothyroidism slows the reaction time and decreases muscular strength, it is an ideal endocrinopathy to insure "accidental" death in the wild.

One might consider what evolutionary advantages accrue from having each adult die shortly after a few reproductive cycles. Compared to small animals, such as insects, large animals, such as mammals, can have only a relatively small number of their species in a given area. For the sake of rapid evolution, it is desireable to have the maximum number of genetic combinations in the shortest time. However, it is useless to perform these experiments of nature unless the new life forms can survive. Unless they die or weaken and can be readily killed, long-lived adults will be pitted in an unequal struggle for survival against their young, weaker progeny. Since the number of the matings is not as important as the number of matings between animals with different gene pools, it is an advantage to limit the number of reproductive cycles of the specific adults

so the genes of their progeny can mix. Finally, if death were not strictly regulated, some animals of a given species might live long lives and produce a relatively large number of their offspring in subsequent generations. The chances of inbreeding among closely related animals would then increase. This could result in the expression of deleterious recessive genes.

In conclusion, the present theory and the above considerations suggest that while death is a tragedy for the individual, it may have evolved as a precisely timed event that increases the adaptability and hence the viability of the species.

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