

Non-enzymatic molecular damage as a prototypic driver of aging

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The chemical potentialities of metabolites far exceed metabolic requirements. The required potentialities are realized mostly through enzymatic catalysis. The rest are realized spontaneously through organic reactions that (i) occur wherever appropriate reactants come together, (ii) are so typical that many have proper names (e.g. Michael addition, Amadori rearrangement, and Pictet-Spengler reaction), and (iii) often have damaging consequences. There are many more causes of non-enzymatic damage to metabolites than reactive oxygen species and free radical processes (the “usual suspects”). Endogenous damage accumulation in non-renewable macromolecules and spontaneously polymerized material is sufficient to account for aging and differentiates aging from wear-and-tear of inanimate objects by deriving it from metabolism, the essential attribute of life.

The idea that aging results from the gradual accumulation of molecular damage is deeply rooted in the aging research field (1–3), although it can appear in verbal disguises so different as to seem conceptually independent. However, damage is implicit to DNA in the somatic mutation theory of aging (4), to the extracellular matrix proteins in the crosslinking theory (5), and to phospholipids in the membrane theory (6). The free-radical theory implies that reactive oxygen species (ROS)⁴ are responsible for damage (7), and the carbonyl-stress theory blames free carbonyls for it (8, 9). With regard to the last two theories, the former, which is based on the ideas of D. Harman (10), celebrated its 60th anniversary last year and remains the most influential in the “damage field,” and the latter (9) is its

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⁴ The abbreviations used are: ROS, reactive oxygen species; SOD, superoxide dismutase.

extension insofar as it attributes the origin of many of the most noxious molecular species to the free-radical oxidation of metabolites initially devoid of highly reactive carbonyl moieties (11). Being traceable back to serendipitous findings, these concepts as of today remain empirical.

The dark side of metabolism

A theoretical approach to molecular damage may be derived from comparing a metabolic map (e.g. IUBMB-Nicholson Metabolic Maps, Minimaps, & Animaps website) or a database of metabolic pathways (e.g. Refs. 12 and 13) with a comprehensive manual of organic chemistry (e.g. Ref. 14). It may be inferred that in many cases of inevitable but reversible imine (Schiff base) formation between amines and carbonyl compounds, the structures of the interacting molecules provide for the migration of the double bond of the imine moiety away from it. This will convert the unstable C=N (imine) bond into the stable C–N bond and thus create a sink for the initial reactants. The migration mechanism may involve the so-called Amadori rearrangement when an aldose reacts with the amino group of, for example, ethanolamine, an amino acid, or a nucleic base (Fig. 1A). Another double-bond migration mechanism is a sigmatropic rearrangement, such as when ethanolamine reacts with retinal (Fig. 1B). Notably, the products may be able to bind other molecules, e.g. by imine formation (Fig. 1A) or by the Michael addition mechanism (Fig. 1B), providing for still further spontaneous transformations, such as involving another chemical classic, the Mannich reaction (Fig. 1B). Another case of transformation of the unstable C=N bond into the stable C–N bond is when an arylethylamine interacts with an aldehyde or ketone via the Pictet-Spengler reaction (Fig. 1C), which is widely used in organic synthesis to obtain polycyclic compounds (15).

Looking at a metabolic map, one can notice many such inherently reactive metabolite pairs. However, few if any of the pair members are connected in the map by arrows marked with enzyme classification (EC) numbers. In some cases, the members are confined to different tissues or compartments; however, they often also occur in the same place and are thus doomed to react. That is how cytotoxic Pictet-Spengler products can be formed from biogenic amines and aldehydes (16, 17). The same is true for 5-S-cysteinyl dopamine, which is formed in the brain from dopamine-derived quinones via nucleophilic thiol addition (18) (Fig. 2A), and for many other compounds such as those in Figs. 1 and 2 and in the chemical damage database CD-MINE (19).

Because of metabolic requirements, many inherently reactive moieties become even more reactive when they are attached to specific molecules known as coenzymes. Examples are the acyl-CoA species (Fig. 2B), which spontaneously acylate primary amines (20, 21). Similarly, acyl phosphates react non-enzymatically with amines (22).

Figs. 1 and 2 show that, in a metabolic system, not only spontaneous decay and degradation reactions, such as hydrolysis, oxidation, and racemization, but also spontaneous multistage

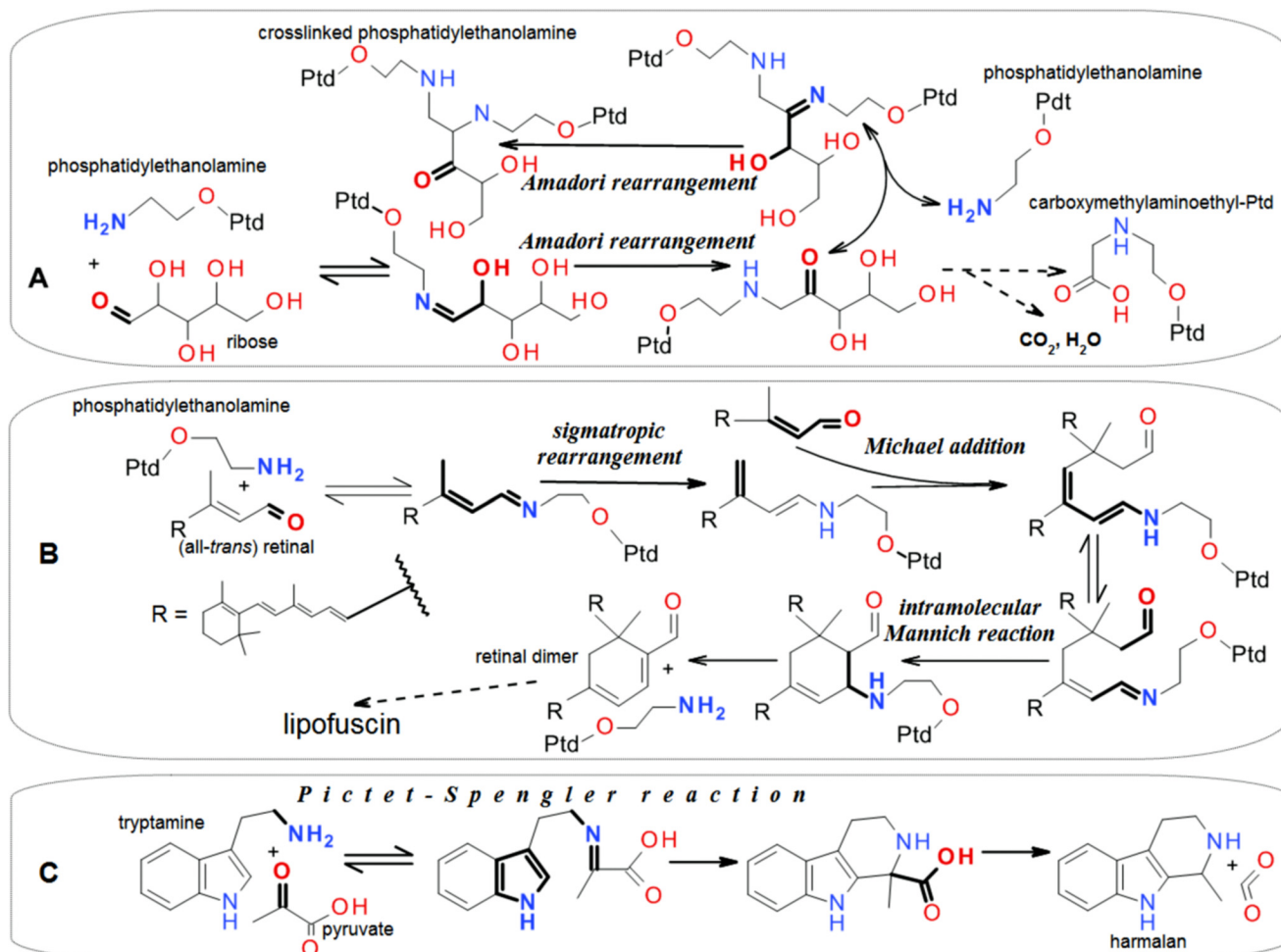


Figure 1. Examples of non-enzymatic synthesis of complex compounds from simpler metabolites via Schiff base formation followed by double-to-single carbon-nitrogen bond conversion. The starting reactive moieties are highlighted with *thick bonds* and *bold characters*. Any other metabolically relevant structure or atom may replace a non-highlighted part, including phosphatidyl (indicated by *Ptd* in *A* and *B*). *A*, ribose exemplifies any monosaccharide. The amino group may be on a phospholipid (23), an amino acid (24), or a nucleic base (25), either free or included in a biopolymer, which may become cross-linked by such modification. *B*, see “Physiological implications of endogenous chemical damage” for comments. See Refs. 26 and 27. *C*, tryptamine exemplifies any indoleamine, and pyruvate represents any carbonyl-containing compound, including saccharides. With catecholamines instead of indoleamines, the Pictet-Spengler reaction yields tetrahydroisoquinolines, whose neurotoxicity is implicated in brain aging (16, 17). Figures were drawn using MarvinSketch 16.5.2.0 (ChemAxon).

synthetic processes take place (23–32). Can the products formed in this way be regarded as metabolites *sensu stricto*? They are not generated by enzymes, are not used purposefully, and are often hazardous. One way to view them is as damaged metabolites (33, 34). For example, 5-*S*-cysteinyl-dopamine is a damaged form of cysteine or dopamine. A related way to conceptualize this phenomenon is to view it as a sort of “underside” of metabolism or “parametabolism” (35, 36). In this view, 5-*S*-cysteinyl-dopamine and tetrahydroisoquinolines are the parametabolic products of catecholamine metabolism. A conceptually similar but more general approach is to regard such unwanted products as a manifestation of the imperfectness of metabolism and its components, which together produce deleterious effects at all levels of biological organization. The totality of such effects has been described as the “deleterium,” which expands with age and represents the biological age of an organism (1).

A complementary point of view comes from the notion that life must have developed from the physicochemical world. It is

true that molecules whose properties are useful for biological functions were selected for the metabolism of living organisms. However, the properties of such molecules are not limited to those required for life. For example, only the cyclic forms of sugars, such as glucose, are used by biological systems and thus have steric compatibility with the active centers of sugar-metabolizing enzymes. However, free sugars in solution consist of an equilibrium mixture of cyclic and linear forms. The linear carbonyl form can react with free amine groups to form Schiff bases, and then, via double-bond migration (Fig. 1), the damaged metabolites resulting from such interactions can leak into the parametabolic mess beneath the metabolic order and add to the deleterium.

One way to increasing the deleterium is by the spontaneous polymerization of damaged metabolites, such as catecholamine-derived quinones (Fig. 2A). In reality, such polymerization occurs in a milieu abundant in proteins, which are included in the resulting agglomerates, wherein they become covalently modified and misfolded and thus made prone to

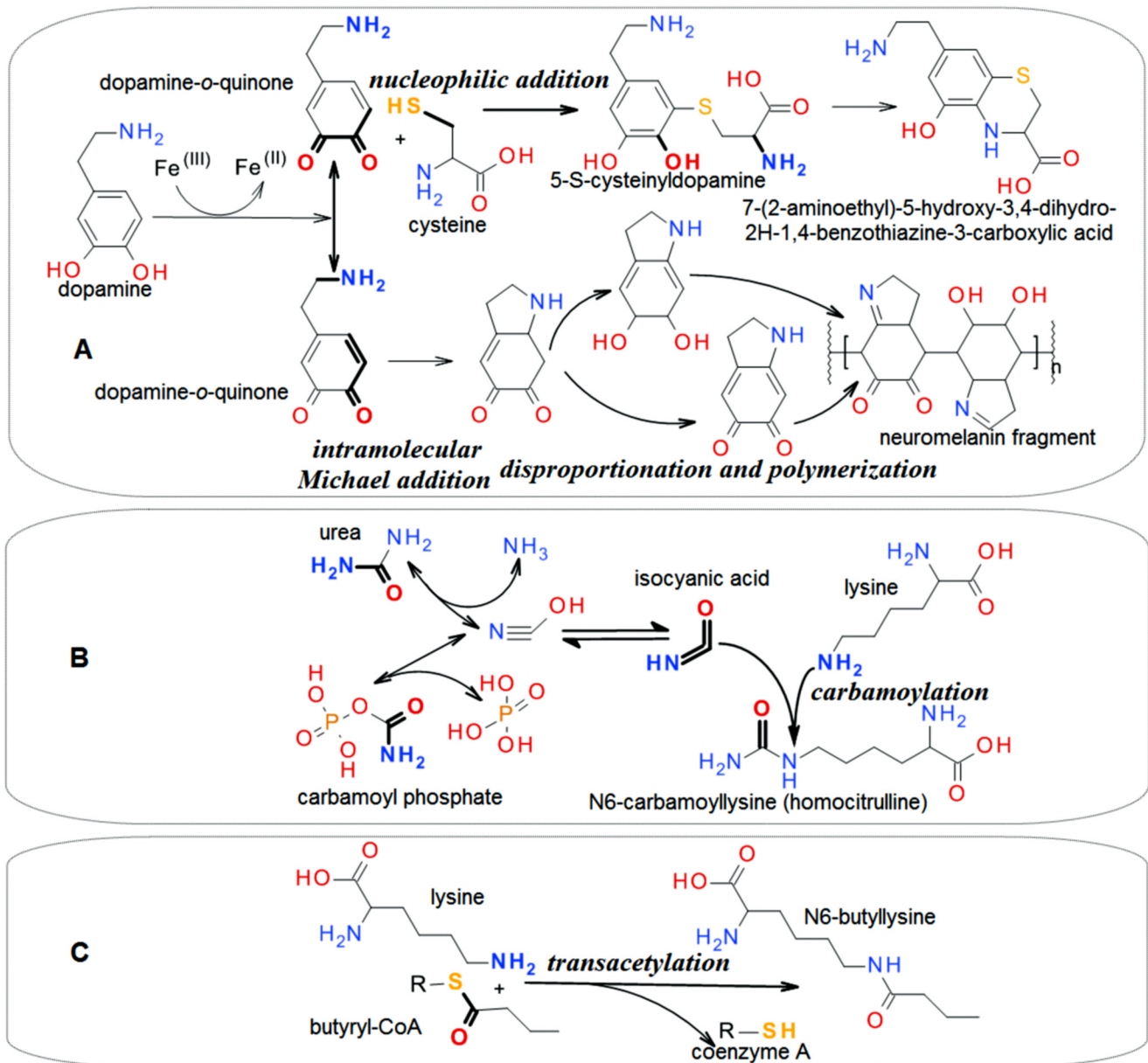


Figure 2. Examples of non-enzymatic synthesis of complex compounds from simpler metabolites without Schiff base involvement. See Fig. 1 for general comments. *A*, dopamine exemplifies any catecholamine. Quinone formation is possible without oxygen involvement, being driven by ferric iron reduction (28). Cysteine may be a free amino acid or a part of glutathione or protein. 5-S-Cysteinyl dopamine is mostly protein-bound, whereas the free cysteine gives rise to cytotoxic dihydrobenzothiazine derivatives (18, 29). *B*, carbamylation of any amino-group bearer is possible. Only the homocitrulline level in human skin has been shown so far to increase with age (30). *C*, high reactivity of thioesters is long acknowledged in chemistry. Their ability to acylate cellular proteins non-enzymatically is reported in several recent publications (20, 31, 32).

aggregation. Altogether, this leads to the accumulation of polymers of (damaged) metabolites associated with protein aggregates in the form of lipofuscin (37), neuromelanin (38), and other forms often referred to as waste.

Countermeasures

Several reasons may be envisioned for the need to control parametabolic short circuits, leaks, and waste heaps. The first is to reduce the loss of resources caused by fluxes into unwanted products. In this case, investment in measures to counter the losses is constrained by the need to avoid consuming more resources than are saved.

A second reason follows from the ability of some damaged metabolites to interfere with legitimate metabolic processes by replacing normal metabolites in their interactions with enzymes, transporters, or receptors whose specificity is imperfect. Such cases may warrant extra investment in countermeasures because the products are actively harmful as opposed to merely useless.

A third reason is because some damage-prone metabolites are present in cells not only as free entities but also as monomers of macromolecules whose turnover rate is low, making the elimination rates of damaged metabolites incorporated in macromolecular structures lower than those of their free counter-

parts. In such cases, parametabolic products may eventually reach higher steady-state levels than are achievable when all reactant species are free. The amounts of macromolecules modified in this way may increase enough to produce significant effects.

An extreme of the above possibility is observed when the turnover rate of a macromolecule is so low that its half-life is comparable with that of a cell containing the macromolecule or the organism itself (39, 40). In such a case, the level of modified macromolecular species will gradually increase during the whole lifespan of their host and unavoidably compromise vital functions. If this is not aging, then what is?

The most straightforward way to cope with such damage is to dilute it by increasing the normal biomass and/or to ensure the turnover of the sum of the normal and abnormal biomass during cell turnover (41). This mechanism operates by default in populations of unicellular organisms and in metazoans whose cell populations are all renewable, *e.g.* the coelenterate *Hydra vulgaris*, which exhibits no manifestations of aging (42). However, this does not eliminate the need to cope with the loss of resources via parametabolic products whose turnover rate is high or with the adverse effects of the steady-state levels of damaged metabolites. For this, mechanisms that have been termed “metabolite damage pre-emption” and “metabolite repair” (33, 43) have evolved. The latter are often coupled to ATP hydrolysis and/or consumption of reducing equivalents and thus are resource-intensive.

Strategies employed by cells to cope with parametabolic damage, including those of the metazoans that benefit from their non-renewable cell populations, are shown in Table 1 (41–55).

Other important aspects of counteracting the forces that produce chemical damage to living bodies are discussed in the next section in relation to the disposable soma theory of aging.

Evolutionary implications of endogenous chemical damage

One conclusion from the above is that many metabolites prone to adverse interactions, including such pivotal molecules as carbohydrates, urea, and acyl-CoAs, were seamlessly incorporated into metabolism at early stages of evolution under conditions that made the accumulation of their parametabolic products negligible because of turnover and dilution. Many known non-enzymatic interactions were at work prior to the advent of aerobic forms of life. For example, methylglyoxal, which participates in certain metabolic pathways in bacteria, performs no function in other prokaryotes. Nevertheless, it is generated in them and in eukaryotes as a by-product of glycolysis. In either case, it can interact spontaneously with nucleophiles, including those incorporated in proteins and nucleic acids, all this without the involvement of molecular oxygen and/or ROS (56).

Although the range of parametabolic reactions enormously expanded with the advent of metabolic use of molecular oxygen and its derivatives, the unwanted consequences of metabolism were never, and are not today, limited to ROS-related reactions (11, 57). The examples in Figs. 1 and 2 and in Table 1 are deliberately chosen as cases where the initiation of damage by ROS or nitric oxide and its propagation by free radicals are not

essential. These examples still suggest that the deleteriousness of a system where these processes take place will continuously increase, although its density may be maintained and even decreased by dilution due to growth and proliferation. ROS can contribute to the final picture quite appreciably; however, they are neither necessary nor sufficient to make a system age.

The significance of non-enzymatic processes changed dramatically when it emerged in the course of evolution that the presence of non-renewable cells (*e.g.* in the brain) and extracellular matrix components (*e.g.* in the endoskeleton) could have adaptive value for populations to the extent that their benefits outweighed the adverse effects of damage accumulation in individual bodies. Thus, the advent of non-renewable structures as means of protection from exogenous hazards was associated with the actuation of the hidden endogenous damage potential, which previously could be neutralized by dilution and turnover (36) and pre-emption (34).

An influential evolutionary concept directly relevant to endogenous chemical damage is the antagonistic pleiotropy theory of aging (58). Antagonistic pleiotropy refers to the ability of a mutation to produce positive effects on some vital function at the expense of negative effects on another function. Specifically with regard to aging, it was suggested that if a mutation that offers some advantage early in life confers a disadvantage at ages so advanced that they are virtually unachievable in the wild, the mutation must be supported by natural selection because, at the population level, its later-acting disadvantageous effects are outweighed by the earlier advantageous ones. As a result, the vitality of aged organisms must be compromised by the late disadvantageous effects of the genes whose early advantageous effects increase the chance for organisms to survive to the ages of manifestation of their disadvantageous effects.

Some of the concerns about the antagonistic pleiotropy theory include the actual identity of genes that produce early beneficial and late detrimental effects and of mechanisms that switch the effects from beneficial to detrimental (59). However, the above view on the causes of damage to metabolites and macromolecules changes the entire dispute. Any gene whose protein product, such as an enzyme, is involved in the production of a vital metabolite capable of unwanted interactions fits the concept of antagonistic pleiotropy insofar as it produces the direct beneficial effects on vitality through the metabolic functions and the adverse pleiotropic effects through the excessive chemical potentialities of its metabolic product. The adverse effects gradually increase with the accumulation of the results of unwanted interactions, such as the agglomerates of their products and/or damaged slowly turning-over proteins and nucleic acids. That is, the adverse pleiotropic effects are not late-acting, as they are commonly thought to be, but are cumulative (36). Another corollary is that there are no, and there never were, genes that are completely free of adverse pleiotropic effects. Antagonistic pleiotropy responsible for aging results from gene activity in the context of the whole system of interacting genes and their products rather than from any of its specific components taken separately at different points in the lifespan. Therefore, the diversity of damage forms will always exceed the number of protecting mechanisms, and, for non-

Table 1
Mechanisms that help cells to cope with the endogenous chemical damage

Option	Examples	Costs and limitations
Dilution	Any growing cell population (41)	Increases in biomass/size/amount must be unlimited. This is incompatible with biological strategies that rely on fixed-size non-renewable structures, such as those present in the brain and endoskeleton.
Total turnover	<i>Hydra vulgaris</i> (42)	Available resources are spent for <i>de novo</i> synthesis of turning-over material. This is incompatible with benefits afforded by the presence of non-renewable cells.
Uneven partition of damage	Damage segregation upon division in <i>Escherichia coli</i> and damage bias to mother cell upon budding in yeast (44)	Continuing cell proliferation is required to maintain tolerable proportions of relatively more and less damage-loaded cells.
Pre-emption ^a	Glyoxalases (Glo I and II) in concert with glutathione convert reactive methylglyoxal (MGO) to inert D-lactate (45).	Resources are spent for the synthesis and turnover of pre-emption enzymes. Notably, Glo I is among the 5% most abundant proteins in many human tissues (PaxDb 1855892), and so is triose phosphate isomerase (PaxDb 1843191), whose ability to mitigate MGO production is limited by the diffusion rates of its substrates (45).
Directed ^a overflow	Accumulation of hazardous intermediates of riboflavin biosynthesis is limited by diverting them to an alternative pathway (46).	The level of the diverted metabolite must not fall below that required for basal metabolic functions.
Trapping	Methylglyoxal is trapped by the dipeptide carnosine (47).	Carnosine is a "suicide" trapping agent; therefore, resources are spent for its regeneration.
Repair ^a	Amadori products and methylglyoxal adducts are removed from proteins and free amino acids by fructosamine-3-kinase (48) and DJ-1/Park7-type deglycosylases (49), respectively. SIRT3 sirtuin removes acyl groups from proteins damaged non-enzymatically by acyl-CoAs (21). DNA repair	Resources are required for the synthesis and turnover of DJ-1, which is among the 5% most abundant proteins in human tissues (PaxDb 1852305). Fructosamine-3-kinase consumes ATP. NAD is consumed in the deacetylation reaction.
Clearance	Ubiquitin-proteasomal system (51) Chaperone-mediated autophagy (52) Microautophagy (53) Macroautophagy (3)	DNA repair mechanisms are so complex and resource-intensive that their affordable repertoire critically depends on available resources (50). Ubiquitination consumes ATP. These mechanisms are thought to reallocate cell resources by their degradation and reuse according to changing demands and, as such, may produce no waste if an undamaged material is processed. Under stable conditions, they ensure the total turnover of cell constituents and, thus, the maintenance of a tolerable stationary level of damage outside of autophagosomes and lysosomes. However, some forms of damage, such as shown in Figs. 1 and 2, can resist degradation and accumulate with time in the form of lipofuscin.
Excretion	For the passage of water-soluble metabolites across cell membranes, transporters such as ATP-binding cassette (ABC) transporters (54) or solute carrier (SLC) proteins are required (55). The same must be true for hydrophilic damaged metabolites and the products of their trapping and of damage preemption.	ABC transporters consume ATP. Many SLCs depend on transmembrane gradients generated by energy-consuming mechanisms. Among about 400 known human SLC genes, three-quarters are not yet associated with a known transported substrate (55). Some of these orphan SLCs may be involved in the excretion of endogenous chemical damage products.
Disposal	Catecholamine-derived quinones polymerize spontaneously to yield neuromelanin (38).	Immediate damage by highly reactive monomers is reduced at the expense of delayed adverse effects of increasing space occupied by relatively inert but bulky polymers.

^a Other examples may be found in Ref. 34.

renewable systems, the consequent cumulative damage will necessarily increase, manifesting as aging.

A common misconception worth mentioning is that damage results from “stresses,” such as oxidative stress (60) and carbonyl stress (2, 9). Stress is thought of as a condition wherein the effects of damaging factors surpass the ability of the system to protect itself from damage. However, damage occurs regardless of stresses, which merely modify the rate and composition of damage accumulation. For instance, in mammals under conditions assumed as basal, the gaseous products of lipid peroxidation are still exhaled (61) and damaged nucleosides are excreted (62).

It is possible, in principle, to slow down damage accumulation by supplementing pre-emption, repair, and elimination of damage to metabolites with repair of damage suffered by non-renewable proteins (48, 63) and DNA (64). However, increased investment into self-maintenance increases its total cost at the expense of other functions essential for survival of a population. This is what the “disposable soma theory of aging” is about: protection from damage that causes aging is limited by the need to allocate a part of the available resources to reproduction (65).

An important but largely overlooked question related to this theory is the quantitation of the energetic cost of damage to metabolites and macromolecules and its repair or pre-emption (including the costs of producing the enzymes that do nothing except protect from damage), particularly relative to the costs of other “maintenance” processes such as macromolecule turnover and sustaining chemical and ion gradients (66). A part of the problem is that the overall costs of maintenance are poorly understood and handled as a “black box” in current metabolic models rather than being partitioned among biochemical processes (67, 68). Another challenge is that good kinetic data for spontaneous reactions under *in vivo* conditions are very often lacking, making it hard to estimate the rates of formation of damaged metabolites. Special measurements under conditions that mimic those *in vivo*, as in Ref. 69, are required for that. Nevertheless, there is reason to think that parametabolism contributes significantly to maintenance costs, as, for instance, in the case of *S*-adenosylmethionine, which suffers relatively high rates of spontaneous racemization as well as cleavage and hydrolysis (70) and is energetically expensive to repair or replace (34).

However, it is still unclear whether the above total costs amount to, say, 50, 10, or 1% of the total energy flux. The lowest of these estimates would make the cost of coping with endogenous damage almost irrelevant, thus making the disposable soma theory less relevant, too.

Both the antagonistic pleiotropy and the disposable soma theories in their classic forms are based on considerations derived from evolutionary theory, population genetics, and physiological and ecological tradeoffs. These theories either treat the chemical constituents of living organisms as if they impose no constraints on “molding of senescence by natural selection” (71), or admit such constraints but treat them as resulting from unfortunate “frozen accidents” that were missed by selection. Why selection missed them is then explained in terms of tradeoffs or other balances, which is circular reasoning. This approach is comforting in that accidental flaws (and

by inference aging) are more likely to be amenable to elimination than are essential features. However, “ignorance of the law excuses not.” By the laws of chemistry, carbonyls react with primary amines. This is not suggested by evolutionary theory and cannot be eliminated by natural selection. Water’s capability of adding to carbon-carbon double bonds will be realized upon whatever opportunity, such as interacting with NAD(P)H to form NAD(P)H hydrate. Shifting the equilibrium between dysfunctional NAD(P)H hydrate and functional NAD(P)H toward the latter is possible at the expense of ATP hydrolysis by the repair enzyme NAD(P)H-hydrate dehydratase (72).

Another important aspect of chemical damage to biochemical systems is that any biochemical means devised by evolution to cope with such damage expands the range of possible interactions, including adverse ones. Thus, superoxide dismutase (SOD), which disposes of harmful superoxide, yields hydrogen peroxide, which is prone to forming hydroxyl radical via the Fenton or iron-catalyzed Haber-Weiss reaction. Therefore, SOD must be coupled with catalase or peroxidase. Increasing Cu,Zn-SOD expression may result in increased oxidative damage, and in some experimental settings, mimics phenotypes observed in Down’s syndrome patients (73). Cu,Zn-SOD itself is inherently prone to aggregation (74), so that the mutant forms that are responsible for the hereditary amyotrophic lateral sclerosis merely exaggerate an existing tendency. From this perspective, it is no wonder that the incidence of the sporadic adult-onset forms of this disease is above zero.

Physiological implications of endogenous chemical damage

A good case for applying the ideas discussed above to a specific situation is provided by bisretinyls, the major constituents of lipofuscin that accumulate in the pigmented epithelium of the eye. Bisretinyls, such as retinal dimer (Fig. 1B), are byproducts of visual cycle biochemistry (75). 11-*cis*-retinal bound to the ϵ -amino group of lysine 296 of opsin in the outer segments of photoreceptor cells is converted to all-*trans*-retinal upon accepting a quantum of light. Although the imine moiety of retinal bound to opsin is formed in a non-enzymatic reversible manner, retinal is kept in its place by non-covalent interactions of its 11-*cis* configuration with properly arranged side chains of the other amino acids of rhodopsin. The all-*trans*-retinal does not fit opsin structure and is expelled from it. On the one hand, this is associated with opsin conformation changes triggering signal transduction via G-proteins. On the other hand, liberation from opsin enables retinal to form Schiff bases with amino groups, including those of phosphatidylethanolamine in photoreceptor cell membranes. To avoid this, all-*trans*-retinol dehydrogenase consumes NADPH to reduce all-*trans*-retinal to the less noxious all-*trans*-retinol. Retinol is transferred to pigmented epithelium cells, where it is converted to 11-*cis*-retinal. The latter enters receptor cells and forms the Schiff base with lysine 296 of opsin there.

Without delving into important details and conflicting views, it is sufficient in the present context to point out that the functional demands of light perception ensure that the aldehyde retinal is constantly present in a free form in an environment rich in ethanolamine moieties. The result is that the reversible

Schiff base formation from an ethanolamine moiety and retinal can be followed by the irreversible addition of another retinal to the product, and after a series of further rearrangements, by the formation of retinyl dimer (Fig. 1B) and a host of related compounds accumulating in photoreceptor membranes, which are constantly shed off to be phagocytized by pigmented epithelium cells. The poorly degradable retinal dimer and related products form lipofuscin deposits in pigmented cells and thus increase the risk of macular degeneration, the most common form of age-related vision loss.

Several lessons follow from the above case. First, damage accumulation results from normal functions, and the pathways of damage formation may become clear only after the molecular details of normal functions become known. Second, damage manifests itself in a functionally significant manner at ages rarely achievable in the wild under the conditions in which the species in question evolved. Therefore, there was no selection pressure toward the prevention of accumulation of this sort of damage. However, there was pressure toward preventing any immediate damage, even at the expense of later adverse consequences. In fact, lipofuscin accumulation in pigmented epithelium is a consequence of clearing of photoreceptor cell membranes from damage caused by retinal liberated in the course of light perception. Third, via a series of transitions through rapidly turning-over cell constituents, damage finally accrues as a slowly turning-over material in the non-renewable component of a functional system where the deposits of damaged metabolites accumulate. Fourth, the pathways from metabolite damage to the accumulation of agglomerates of damaged metabolites and, further on, to age-associated functional decline may be deciphered at a resolution approaching specific chemical interactions between metabolites, which leaves no place for speculations regarding the causes of aging.

Lipofuscin abundance in steroid hormone-producing organs, such as testes, ovaries, and renal cortex, highlights other important aspects of molecular damage and its evolutionary origin (or neglect?). The latest evolutionary extensions of steroidogenic pathways yield products destined for secretion as hormones even though their chemical properties make them pseudo-substrates for some upstream steroidogenic enzymes. By binding to the active centers of these enzymes, the end products of steroidogenesis, such as testosterone (76) or cortisol (77), initiate one-electron oxygen reduction but do not react with the resulting ROS, which are released and damage everything in their vicinity, starting with the enzymes themselves.

The maximal steroidogenic capacities of testes, ovaries, and adrenal cortex markedly decrease during aging; however, their actual productivities decrease much less (78). A likely explanation of this discrepancy may be the observation that the negative feedback regulation of steroidogenic tissues is mediated by central catecholaminergic neurons, whose functions become compromised with aging (79). Catecholamines and their metabolites and parametabolites are prone to Pictet-Spengler-type interactions yielding products implicated in age-associated parkinsonism (17), and are subject to oxidation yielding neurotoxins (80), including quinones (81). These quinones in turn polymerize (Fig. 2A), leading to neuromelanin accumulation in catecholaminergic neurons present in brain structures

whose deterioration is implicated in emotional and cognitive disorders (82, 83). The production of certain steroids may actually increase with aging in some animal species (78, 84), probably due to the release from inhibition mediated by catecholaminergic mechanisms, which may overcompensate for the decreased maximal steroidogenic capacity.

Altogether, such relationships may generate species-specific patterns of age-associated physiological changes, giving the appearance of a programmed process. However, such patterns are a sort of quasi-program formed as a by-product of genuine programmed (genetically determined) functions whose operation generates noxious parametabolic products (35). As a result, the functions of even the renewable components of a system will be compromised during aging by their milieu, which becomes progressively non-optimal because of quasi-programmed changes in the non-renewable components.

Concluding comments

Recent advances in the metabolomics of aging (85–90) defined patterns of age- and disease-specific changes in the levels of metabolites, *i.e.* low-molecular-weight, enzymatically generated compounds used in biological systems. Such compounds have specific places in metabolic maps, databases, and models of metabolism (12, 13, 91). These resources, however, omit the many cellular compounds that, like those in Figs. 1 and 2, are formed via chemical side reactions. The recently launched chemical damage database project CD-MINE (19) sets out to fill this gap.

Notably, spontaneous chemical reactions between metabolites are often labeled with proper names, such as Schiff, Pictet-Spengler, Amadori, Mannich, or Michael, just because they are typical and will take place wherever the respective reactants come together. Thus, from the chemical point of view, a metabolic system cannot but be plagued with numerous short circuits, leaks, and other adverse concomitants of metabolism.

Unwanted reactions of this sort give rise to diverse damage products that increase in number and abundance with age and are adjusted (with regard to both composition and rate of increase with age) by interventions that affect lifespan (88). These reactions in their entirety are sufficient to cause what is generally termed aging.

Logically, any pursuit of what aging is must end with an explanation (the *explanans*) that resides outside of what has to be explained (the *explanandum*). In this Minireview, we root out the cause of aging in the unwanted, “surplus-to-metabolic-requirements” **chemical** properties of the constituents of **biological** systems. This approach – unlike current evolutionary theories of aging – takes the *explanans* out of its *explanandum*. An interface between the two is metabolism – the quintessential attribute of life making biological aging different from the wear and tear observed in inanimate things. Abrogation of many of the alleged individual causes of aging (*e.g.* damage to DNA (92) or damage by free radicals) will only modify, but will not stop aging. As to the endogenous chemical damage, to abrogate it is the same as to abrogate metabolism, *i.e.* life itself.

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