Series
DOI 10.1007/s12038-011-9167-1

What history tells us XXVI. From Mechnikov to proteotoxicity: Ageing as the result of an intoxication

MICHEL MORANGE
Centre Cavaillès, CIRPHLES USR 3308, Ecole normale supérieure, 29 rue d'Ulm, 75230 Paris Cedex 05, France

(Fax, +33-144-323941; Email, morange@biologie.ens.fr)

1. Introduction

One of the potential interests of historical studies is to outline the similarities between recent models, theories and interpretations, and those of the past. The purpose is not to deny recent models their value, but to help present-day scientists to see the difficulties, and possibly the limits, of the models and theories they propose.

One interesting example of a theory that has been favoured, criticized and recently resurrected in a new form is the vision of ageing as the consequence of a process of intoxication, of a poisoning of the organism.

Many different successive models have been proposed to explain the origin of ageing. Most of these models conceive it as the result of a decrease in the capacity of organisms to face the challenges coming from the environment: decrease in the immune response of the organism towards infectious agents, in the power of cells to divide and for the stem cells to regenerate damaged tissues, in the action of hormones (such as the sexual hormones) – an effect initially described by Brown-Séquard, and in the possibility for the organism to repair damage to DNA and proteins.

But some models of ageing have, in contrast, looked for the cause of ageing in the accumulation of toxic substances. Something is present in the aged organism that was absent in youth, and this 'something' poisons the body.

Such a model of ageing is not new. It was proposed by the physician and alchemist Paracelsus in the Renaissance (Koyré 1997). Its first truly scientific expression was provided by Ilya Mechnikov at the beginning of the 20th century (Lellouch 1993). Today, the idea that protein aggregates accumulate in the ageing body and that they poison the cells and organisms in which they are present is finding more and more support among biologists.

2. Ilya Mechnikov: the role of microorganisms in the gut

From his initial study on the causes of hair whitening and his observation of old parrots in 1901–1902, Mechnikov devoted a huge part of his research to a description and explanation of the ageing process, and published various books in which the main results are reported and the philosophical consequences of these discoveries discussed. I will use two of these books to summarize the conceptions of Mechnikov: *The Nature of Man* (1903) and *The Prolongation of Life: Optimistic Studies* (1907).

The personal reasons for this work have often been discussed. It has been seen as the natural interest that elderly scientists (and nonscientists) show in philosophical issues. This interest was also the prolongation of the studies devoted by Mechnikov to infections, and to the role of phagocytosis (Tauber and Chernyak 1991). Mechnikov had also been shaken by the critique addressed by his former fellow countryman, Lev Tolstoy, to scientists, and in particular to biologists, to leave aside the issues that are of the highest value for mankind, among which is the problem of ageing. Like most biologists of his time, Mechnikov favoured a comparative approach to this question. He showed the diversity of situations occurring in the living world – from animals living for a few hours to trees that were thousands of years old, but also underlined the role

Keywords. Intoxication; Mechnikov; neurodegenerative diseases; protein aggregation; proteotoxicity

that food had on the duration of life. A more precise comparison within vertebrates, between birds and mammals, allowed Mechnikov to confirm the relation between the duration of life and the size of the animals, but also to notice interesting exceptions. The latter seemed to be related to the size of the distal part of the gut: the more it was developed, the shorter was life. Since the gut is filled with microorganisms, Metchnikov deduced that the toxins produced by these microorganisms were responsible for the accelerated ageing process. The consequence was, for Mechnikov, that strict control of the nature of the diet, and of the microorganisms present in the gut, was the best way to delay ageing.

It could not, however, prevent normal ageing, a process also seen by extension as the consequence of the production of toxins (poisons) within organisms. Mechnikov rejected the hypothesis that ageing was the simple consequence of the decrease in the proliferative power of cells. Toxins were responsible for atherosclerosis; they also activated the phagocytes, which led to the destruction of body tissues.

The parallel established between ageing and intoxication was supported by three additional arguments. The first was the relation between natural death and sleep. Both processes looked very similar. Since sleep was simultaneously explained by the accumulation of toxins within active organisms, the resemblance between the two processes reinforced the hypothesis that ageing was due to an accumulation of toxins. In the same years, lipofuscin, pigment granules now identified as lipid-containing residues of lysosomal digestion, was discovered by Mülhmann and shown to be present in old tissues: this yellow substance was the visible sign of the intoxication of ageing organisms. In 1912, Alexis Carrel wrongly demonstrated that cells were immortal when they were extracted from the organisms and cultivated on plates (Carrel 1912). The observations of Alexis Carrel, in particular the demonstration that the renewal of the cell culture medium was required to maintain cell proliferation even before the nutrients present in it had been consumed, were perfectly compatible with the hypothesis that ageing resulted from an intoxication.

What was retained from the ideas of Mechnikov was the sour milk diet that he proposed. The results of such a diet were not convincing, and the hypothesis of Mechnikov on ageing was rejected. One must nevertheless recognize that the hope to discover a diet likely to prolong life is still alive and well!

3. Protein aggregates in disease and ageing

A unique model has recently emerged to explain the development of the most common neurodegenerative diseases – ataxia, Huntington's chorea, Parkinson's and Alzheimer's diseases: they result from the formation of proteotoxic aggregates. There is an active ongoing debate concerning the precise nature of the toxic entity. Large aggregates are now considered weakly toxic, and dealt with via a detoxification pathway. The focus is on small aggregates, since they are able to act as seeds to initiate the aggregation process in neighbouring cells.

Three partially independent lines of research have extended the involvement of aggregates to the general process of ageing. The first was the relation established between protein aggregation and ageing in the animal that has been the model for molecular studies of ageing – the nematode. Human neurodegenerative diseases were 'reproduced' in these animals. The mutations that had previously been shown to prolong life were demonstrated to delay the formation and growth of protein aggregates. As early as 2003, molecular connections between the pathways controlling ageing and protein aggregation had been established, and these extended to other organisms as well (Hsu *et al.* 2003; Cohen *et al.* 2006; Cohen and Dillin 2008; Cohen *et al.* 2009). These pathways modulate the expression of chaperones, a family of proteins that facilitate protein folding and prevent the formation of aggregates.

The formation of aggregates was also described in organisms that, according to August Weismann, do not age because they have not evolved towards the formation of cells specifically involved in reproduction, with the distinction between somatic and germ cells: yeasts and bacteria. Careful observations showed that aggregates are not equally transmitted to the two daughter cells during division, but accumulate in one of them. The daughter cell containing the aggregates grows and divides at a lower rate (Lindner *et al.* 2008). The extension of the phenomenon of ageing to these unicellular organisms paralleled the attribution to these protein aggregates of a major causal role in the process of ageing.

Drugs like thioflavin T that bind to and prevent the formation of aggregates have been shown to delay ageing in nematodes (Alavez *et al.* 2011). Protein aggregates observed in neurodegenerative diseases are increasingly considered as a visible part of an iceberg formed by the progressive aggregation of proteins with age. These diseases occur late in life, even when they result from genetic mutations. Recent studies have shown that the proteins that aggregate during the normal process of ageing are not different from those present in the protein aggregates observed in neurodegenerative diseases (David *et al.* 2010).

Most authors are very cautious in the interpretation of data. Protein aggregation is described as accompanying ageing, and as a sign of the disruption of the 'protein homeostasis network'. Protein aggregation is considered as a 'paradigm of ageing' (Lindner and Demarez 2009). This does not prevent increasing use of the word 'proteotoxicity' to designate the consequences of this process of aggregation. It is noticeable that the meaning of the word has

progressively shifted from 'toxicity for proteins' to 'toxicity from proteins'.

4. Discussion and conclusions

The hypothesis that the formation of non-functional proteins is the main cause of ageing is not new. It was proposed by Leslie Orgel in 1963 (Orgel 1963) as a reaction against the mutational model proposed by Leo Szilard some years before (Szilard 1959). If the non-functional proteins are involved in fundamental processes such as protein synthesis, this event can have catastrophic consequences.

The causative role of free radical and oxidative stress in ageing was independently proposed by Denham Harman (Harman 1956), and found strong support in numerous observations showing a link between mitochondrial dysfunctioning – which leads to the formation of reactive oxygen species – and ageing, and the beneficial effects of caloric restriction. Proteins being targets of free radicals, it is easy to link the two models.

In both models, protein aggregates form. But they are only the consequences of the initial event – the formation of non-functional proteins. Ageing is a consequence of the decrease in the normal functions of cells and organisms.

In the protein aggregation model of ageing, the explanation is different. The cause of ageing is the formation of aggregates, which poison cells and organisms. Ageing is the result of the formation of new proteotoxic entities.

The present model of ageing by the formation of toxic protein aggregates, and the past model of Mechnikov are very similar. The only difference is that the toxic entity has been identified – protein aggregates – and that their origin is within the organism (except in the case of the prion diseases), whereas for Mechnikov toxins responsible for ageing were both internal and partially external (produced by the microbes present in the gut).

Such 'positive' theories of ageing are of huge interest: they raise the possibility of finding drugs and treatments that block the process of ageing. For Mechnikov, the solution was both in the control of the 'neuronophages' (the macrophages responsible for the elimination of neurons) activated by the accumulation of toxins, and in changing the nature of the microbes in the gut. For today's researchers, the solution is caloric restriction, the use of antioxidant drugs and the development of new drugs to prevent the formation of aggregates or to permit their de-aggregation.

The weakness of any 'positive' model of ageing stems from the difficulty of squaring it with the evolutionary explanations of ageing. If the cause of ageing is so simple, why did natural selection not succeed in preventing this process? If the distal part of the gut, which has no active role in the digestive process, is responsible for the production of toxins involved in ageing, why has this part of the gut not been eliminated? The answer provided by Mechnikov did not really convince. The animals with a long distal part of the gut are fast-running creatures, and such an ability is crucially necessary to escape their predators. Mechnikov had personally observed that these animals were unable to defecate during running. The conclusion was simple: the possession of an elongated digestive track allowed the animal to accumulate faeces, with the resulting production of bacterial toxins and an abbreviated life; it was the price to pay for the ability to run fast.

In current models of ageing by protein aggregation, the situation is not better. Molecular mechanisms targeting protein aggregates do exist: chaperones prevent protein aggregation, or de-aggregate proteins, the proteasome destroys misfolded proteins and protein aggregates, and the process of autophagy is able to eliminate large protein aggregates. The question is, therefore, shifted: why are organisms unable to eliminate these protein aggregates when they age? Maybe, as proposed by the soma-disposable theory of ageing, the reason is that organisms have invested in reproduction, and not in these repair mechanisms. Whatever the final explanation turns out to be, the hope of discovering, in the formation of toxic protein aggregates, *the* cause of ageing has vanished.

Acknowledgements

I am indebted to David Marsh for his critical reading of the manuscript.

References

Alavez S, Vantipalli MC, Zucker DJS, Klang IM and Lithgow GJ 2011 Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan. *Nature (London)* **472** 226–229

Carrel A 1912 On the permanent life of tissues outside of the organism. *J. Exp. Med.* **15** 516–528

Cohen E, Bieschke J, Perciavalle RM, Kelly JW and Dillin A 2006 Opposing activities protect against age-onset proteotoxicity. *Science* **313** 1604–1610

Cohen E and Dillin A 2008 The insulin paradox: proteotoxicity and neurodegeneration. *Nat. Rev. Neurosci.* **9** 759–767

Cohen E, Paulsson JF, Blinder P, Burstyn-Cohen T, Du D, *et al.* 2009 Reduced IGF-1 signalling delays age-associated proteotoxicity. *Cell* **139** 1157–1169

David DC, Ollikainen N, Trinidad JC, Cary MP, Burlingame AL and Kenyon C 2010 Widespread protein aggregation as an inherent part of aging in *C. elegans*. *PLoS Biology* **8** e1000450

Harman D 1956 Aging: a theory based on free radical and radiation chemistry. *Gerontology* **11** 298–300

Hsu A-L, Murphy CT and Kenyon C 2003 Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* **300** 1142–1145

Koyré A 1997 *Paracelse* (Paris: Allia)

Lellouch A 1993 Metchnikoff (1845–1916) and aging. *Hist. Sci. Med. 27* 13–22

Lindner AB, Madden R, Demarez A, Stewart EC and Taddei F 2008 Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc. Natl. Acad. Sci. USA* **105** 3076–3081

Lindner AB and Demarez A 2009 Protein aggregation as a paradigm of aging. *Biochim. Biophys. Acta* **1790** 980–996

Metchnikoff E 1903 *Etudes sur la nature humaine. Essai de philosophie optimiste* (Paris: Maloine)

Metchnikoff E 1907 *Essais optimistes* (Paris: Maloine)

Orgel LE 1963 The maintenance of the accuracy of protein synthesis and its relevance to ageing. *Proc. Natl. Acad. Sci. USA* **49** 517–521

Szilard L 1959 On the nature of the aging process. *Proc. Natl. Acad. Sci. USA* **45** 30–45

Tauber AI and Chernyak L 1991 *Metchnikoff and the origins of immunology* (New York: Oxford University Press)

*e*Publication: 12 November 2011