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# Folates, bacteria and ageing: insights from the model organism *C. elegans* in the study of nutrition and ageing

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The relationship between nutrition and ageing is complex. The metabolism and synthesis of micronutrients within the gut microbiome can influence human health but is challenging to study. Furthermore, studying ageing in humans is time-consuming and difficult to control for environmental factors. Studies in model organisms can guide research efforts in this area. This review describes how the nematode *Caenorhabditis elegans* can be used to study how bacteria and diet influence ageing and inform follow-on studies in humans. It is known that certain bacteria accelerate ageing in *C. elegans*. This age-accelerating effect is prevented by inhibiting folate synthesis within the bacteria, and we propose that in the human microbiome, certain bacteria also accelerate ageing in a way that can be modulated by interfering with bacterial folate synthesis. Bacterial-derived folates do not promote ageing themselves; rather, ageing is accelerated by bacteria in some way, either through secondary metabolites or other bacterial activity, which is dependent on bacterial folate synthesis. In humans, it may be possible to inhibit bacterial folate synthesis in the human gut while maintaining healthy folate status in the body via food and supplementation. The supplement form of folic acid has a common breakdown product that can be used by bacteria to increase folate synthesis. Thus, supplementation with folic acid may not be good for health in certain circumstances such as in older people or those with an excess of proteobacteria in their microbiome. For these groups, alternative supplement strategies may be a safer way to ensure adequate folate levels.

**Keywords: Folates: Folic acid: Ageing: Microbiome: *C. elegans***

### Ageing and unravelling complex interactions with diet and microbiome

Ageing is the largest risk factor for disease[1], and thus, slowing ageing could have a huge impact on health and well-being as well as reducing the burden of an ageing population on health and other public services, while increasing productivity by allowing people to work for longer[2]. It was recently estimated that increasing life expectancy by 1 year would be worth US$38 trillion in cumulative value to individuals[3]. Dietary interventions can help maintain health with age, but there are many unknowns about the underlying mechanisms and, therefore, the best diet to follow. It is known that the gut microbiome has several interactions with our diet and human health that could in principle influence ageing[4], but the complexity of the interactions means that specific mechanisms are difficult to uncover, especially within the timeframe of human ageing[5]. Experiments in rodent animal models can be very informative but are also time-consuming and expensive and have ethical issues. The nematode *Caenorhabditis elegans* in which the

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bacterial diet and the nutrient medium can be precisely controlled provides a model to rapidly test interventions for their ability to slow ageing[5]. Furthermore, the molecular mechanisms of action can be uncovered using the established genetics and other tools developed over decades of research using this model. This review will discuss how research in *C. elegans* has revealed an impact of folate synthesis within bacteria on host ageing and how that might have implications for how folic acid supplements interact with the host microbiome and affect health.

## The key features of the *C. elegans* model

*C. elegans* is a well-established model organism for biomedical research, having revealed some key findings such as the genes for apoptosis, the mechanism of RNA interference and the identity of many genes involved in neurobiology[6]. It has recently been shown that research in *C. elegans* can be effective in drug discovery[7,8]. However, more case studies are needed to demonstrate the material contribution of *C. elegans* research to the development of approved drugs or dietary interventions. *C. elegans* is well established in ageing research and benefits from the fact that it has a lifespan of 2–3 weeks and shows signs of ageing within a week, making experiments fast to perform[9]. Much of what is known about molecular pathways that influence ageing comes from research using *C. elegans*[9]. For example, mutants that disrupt the insulin/insulin-like growth factor signalling pathway were first discovered to influence ageing in *C. elegans*[9]. Disruption of this pathway using genetics has been also shown to increase lifespan in the fruit fly *Drosophila melanogaster* and in mice[9] and is linked to why large dogs with increased Insulin-like growth factor signalling live shorter than small dogs[10]. The pathway ends in the regulation of the DAF-16/FOXO transcription factor. Polymorphisms in human FOXO3A are associated with increased human lifespan, further demonstrating the relevance of this pathway to human ageing[11].

In the wild, *C. elegans* live in rotting vegetation where they feed on multiple strains of bacteria growing within that vegetation[12]. In the lab, the worms are fed with a single strain of *E. coli*, which is grown as a bacterial lawn on a Petri dish with nutrient agar. Using a benign lab strain of *E. coli* as food is the universal method used to culture *C. elegans* in the lab. There are no other microbes present as the worms can easily be made to be germ free. This system can be manipulated by changing the constituents of the agar medium, the genetics of the *E. coli* and the genetics of the worm itself[13]. Compounds that impact the bacteria or the animal can be added to the agar. In some ways, the components system represents nutrients (the agar), the microbiome (*E. coli* or other bacteria) and the host (*C. elegans*). In the wild, *C. elegans* extracts its nutrients from vegetation such as an apple by eating the microbes the apple supports when it rots. Humans also use microbes to extract nutrients from apples, but the microbes are contained within the human gastrointestinal tract.

## Inhibiting folate synthesis in *E. coli* extends *C. elegans* lifespan

In a serendipitous discovery, it was found that a particular strain of *E. coli* with a spontaneous mutation led to an increased lifespan of *C. elegans* when the worms were cultured on it[14]. To find the identity of the mutations, researchers used the observation that the mutated strain had a growth defect on minimal media and conducted a screen to find DNA from the wild-type *E. coli* to find a gene that would restore growth. The mutation was found to be in the gene *aroD*, which encodes an enzyme in the shikimic acid pathway used to make aromatic compounds, such as aromatic amino acids and a few other metabolites. By adding back aromatic compounds to the medium to test which one influenced lifespan, it was discovered that the addition of para-aminobenzoic acid (PABA), the precursor of folate, prevented the lifespan increase in the mutant *E. coli* strain[14]. Thus, while the *aroD* mutation prevented the synthesis of several aromatic compounds, the bacteria could get enough of these compounds from the media to support growth without requiring *de novo* synthesis. However, the prevention of folate synthesis in the bacterial mutant led to the increased lifespan of the animal. To test this hypothesis further, folate synthesis in the wild-type bacteria was inhibited by the sulphonamide drug, sulfamethoxazole (SMX). Treatment with this drug resulted in a dose-dependent increase in *C. elegans* lifespan and thus confirmed that inhibition of folate synthesis in the bacteria can slow ageing in the worm as measured both in lifespan and healthspan[14–16].

## Nutritional requirements for folate

All cells require folate for one-carbon metabolism, a process that makes the building blocks of biosynthesis[17]. *E. coli*, like many but not all bacterial species, makes its own folate[18], whereas animals, including *C. elegans* and humans, obtain folate from their diet or associated microbes. Under the conditions in which *C. elegans* is cultured in the lab, inhibiting *E. coli* folate synthesis to the levels in which lifespan was extended did not prevent growth of the *E. coli* or *C. elegans*, showing that sufficient folate remained[14]. Thus, under standard lab conditions, *E. coli* makes much more folate than they need for growth, and likewise, *C. elegans* absorbs more folate from the bacteria than it needs for growth and reproduction.

## A folate deficiency model in *C. elegans*

Folate deficiency in humans leads to neural tube defects during embryonic development. In order to test what happens when folate uptake is restricted in *C. elegans*, use was made of a mutant in a gene that encodes a homologue

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of the enzyme glutamate decarboxypeptidase GCP2. This enzyme assists folate uptake in the human gut by removing glutamates from polyglutamated folates and thus making the folates more able to be transported into intestinal cells[19]. The mutant (*gcp-2.1*) that lacks this enzyme developed and grew normally, but worms developed abnormally on bacteria made to be low in folate from SMX treatment[14]. This developmental defect was not found when folinic acid or folic acid was added to the media, demonstrating that the developmental defect was caused by a lack of folate, and thus, the combination of the *gcp-2.1* mutant and treatment with SMX constituted a folate deficiency model in *C. elegans*. This deficiency model can be used to titrate folate supplementation because the level of folate supplementation that rescues the developmental defect can be deemed to be at a sufficient level.

## Low folate acts in the bacteria, not in the animal, to extend lifespan

A key question was whether the worms live longer because there is less folate in the bacteria or because there is less folate in the animal. Using a dose of folinic acid that prevented folate deficiency in the developmental model, the worms were supplemented at the same time as inhibiting *E. coli* folate synthesis using the folate synthesis inhibitor SMX[15]. Under these conditions, the worms lived longer, suggesting that lowered folate in the bacteria increased lifespan, not because it reduced dietary folate intake (and thereby lowered folates in the worms) but because it prevented the bacteria accelerating ageing[15]. Bacteria might enhance ageing in a folate-dependent way either by producing specific metabolites or some kind of pathogenic physical interaction with the host. We still do not know how this age-accelerating activity works.

## Translation to humans

Looking back through the literature, it had been shown in 1958 that a folate synthesis-inhibiting sulphonamide very similar to SMX increased the lifespan of mice and rats and even dogs[20]. Again, as we find in *C. elegans*, the lifespan extension was reversed by adding PABA to the food, most likely rescuing folate synthesis in the gut microbes in the animals, though that explanation was not considered in the paper. The study was small and needs to be repeated, but it points to the mechanism of slowing ageing being conserved in mammals. In the gut microbiome, some microbes make folate and others do not have the enzymes to do so, relying on folate from other microbes[18]. The proteobacteria, which include *E. coli* as well as many pathogens, are folate producers, and proteobacteria are associated with conditions like obesity and small intestine bacterial overgrowth (SIBO), in which bacteria colonise the small intestine[21]. SIBO is clinically associated with high plasma folate levels, and this folate may derive from the invading bacteria[22]. Perhaps inhibition of bacterial folate synthesis could help treat the condition, not by killing the bacteria but by making them less pathogenic. It is known that disrupting folate synthesis makes many microbes less pathogenic[23]. Our findings in *C. elegans* have raised a number of possibilities for treating human disease and ageing that need to be investigated by studies in mammalian models and humans.

## Folic acid – a breakdown product that feeds bacteria

In the experiments with the folate-deficient *C. elegans* model, we found that folic acid could not rescue the development defect anywhere near as effectively as folinic acid[15,24]. See Table 1 for the differences between various folates. Folic acid is an oxidised form of folate, which is not found in nature but is more stable than reduced folates, and thus used in supplements and in food fortification. We found that folic acid rescues the folate deficiency, not by directly increasing animal folates but by an indirect pathway whereby a breakdown product of folic acid, PABA-glu, is taken up by *E. coli* via the AbgT transporter and broken down further to PABA. This PABA is then used to synthesise new bacterial folate, which can be taken up by the worm when it eats the bacteria[24]. Interestingly, the PABA-glu breakdown product was found in all sources of folic acid that we examined, including a consumer supplement in which it was present at 3–4% of the intact folic acid[24]. Thus, when people take folic acid supplements or fortified food, they are also ingesting PABA-glu, which cannot be taken up by humans but can be used by many folate-synthesising bacteria.

## Questions still to be answered

We still don't know how inhibiting folate synthesis prevents *E. coli* from accelerating ageing in *C. elegans*. One approach is a genetic screening of single gene mutants of *E. coli* to see which extends lifespan. The hypothesis is that if a gene is responsible for accelerating ageing in an age-dependent manner, the deletion of that gene would make *C. elegans* live longer. We screened over 1000 single gene mutants in *E. coli* and found 9 mutants that did extend lifespan[15]. Two were involved in folate synthesis, and the other seven were in genes with diverse functions. While interesting areas of research, none of them clearly revealed the mechanism by which inhibition of bacterial folate synthesis increased lifespan. Another group screened almost 4000 *E. coli* mutants for those that increased lifespan and found 29 mutants including *aroD* and others involved in folate synthesis, but again, none of the mutants clearly revealed a relevant mechanism[25]. Further work with omics analysis is required to uncover potential metabolites or peptides that mediate the age-promoting effects of *E. coli*. Once these have been uncovered in the *E. coli: C. elegans* system they can be investigated in the human microbiome and tested for correlation with disease.

We do not know what the effect of PABA-glu is on the human microbiome and whether the amounts found in folic acid supplements have a negative effect on health.

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**Table 1.** Folates and related molecules mentioned in the text

| Name | Key structure | Comments |
|---|---|---|
| Folates | Pterin - PABA -$Glu_n$ | General class of molecule, with glutamate chain of varied length. Found in every cell, and thus in the diet, mostly in the fully reduced state (tetrahydrofolate) |
| Folic acid | Pterin - PABA -$Glu_1$ | Fully oxidised form of folate – one glutamate – not found in nature |
| Folinic acid – also known as formyl tetrahydrofolate | Pterin - PABA -$Glu_1$ | A reduced form with a formyl group – found in nature |
| PABA-glu | PABA-$Glu_1$ | Breakdown product of folic acid and other monoglutamated folates |

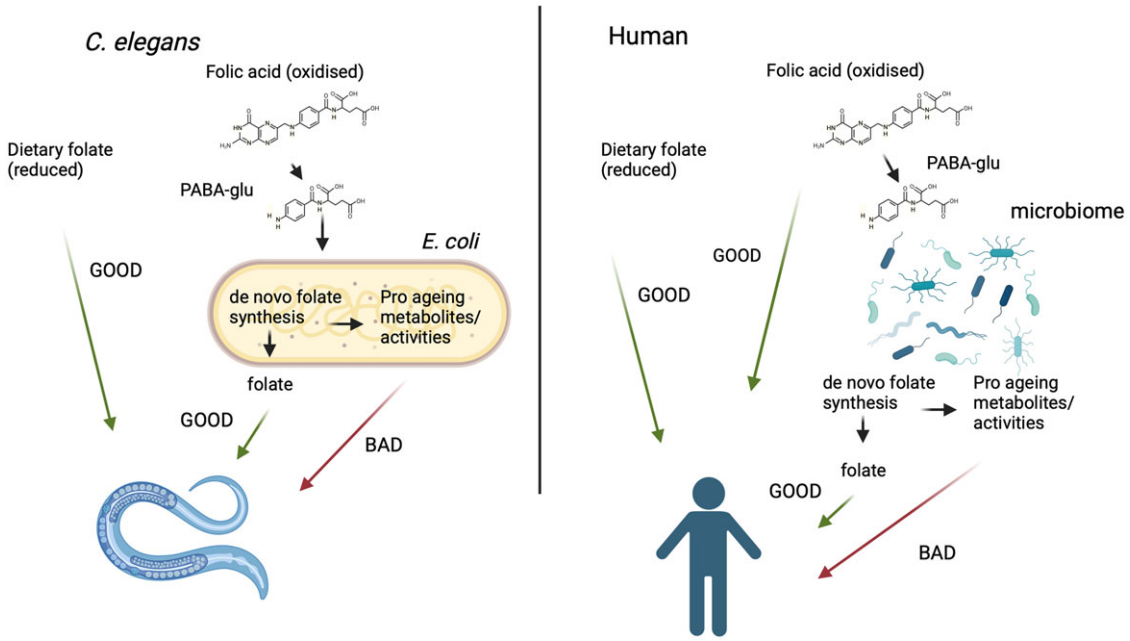


**Fig. 1.** Schematic summarising our findings in *C. elegans* and how it might translate to humans. Dietary folate is required for worm development. Folic acid, an oxidised form of folate not found in nature, is not well taken up by the worm, but the PABA-glu breakdown product can be taken up by *E. coli* and used for *de novo* folate synthesis. Folate generated via this route is beneficial for the worm, but increased folate synthesis leads to metabolites and/or other bacterial activity that accelerates ageing. A similar situation may exist in humans in which folic acid ingestion is likely to increase *de novo* folate synthesis in the human gut microbiome, which, while providing folate to the host, may also have negative effects via the increased production of pro-ageing metabolites or other bacterial activities.

Clinical studies would allow some of these questions to be investigated.

## Conclusions and future perspectives

The findings of this work are summarised in Fig. 1(a). Overall, this research raises the possibility that bacteria may be ageing us and that finding ways to interfere with the age-promoting activity of those bacteria may be a mechanism to slow human ageing and disease progression (Fig. 1(b)).

The challenge is to find a safe way to achieve long-term interventions to slow microbial folate synthesis without compromising safety. With an optimised method of supplementation, perhaps with reduced folates such as folinic acid, it should be possible to provide sufficient folates to the human body while restricting folate supply in the microbiome. A greater understanding of the folate 'economy' in the gastrointestinal tract both in health and disease is required to implement these interventions. An alternative but not mutually exclusive approach would be to intervene in the downstream mechanisms that make microbes enhance ageing, but that requires further understanding to identify and disrupt these mechanisms.

The other major finding is that there are high levels of the breakdown product PABA-glu in folic acid preparations and that this breakdown product can be used by bacteria to synthesise fresh folate. It needs to be established whether this process is occurring in people, if it has any negative consequences on health and whether there are other ways to increase folate levels, especially in the elderly or those with conditions that change the microbiome.

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## Author contributions

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## Competing interests

None.

## References


1. Niccoli T & Partridge L (2012) Ageing as a risk factor for disease. *Curr Biol* **22**, R741–R752.
2. Scott AJ (2023) The economics of longevity – an introduction. *J Econ Ageing* **24**, 100439.
3. Scott AJ, Ellison M & Sinclair DA (2021) The economic value of targeting aging. *Nat Aging* **1**, 616–623.
4. Aggarwal N, Kitano S, Puah GRY *et al.* (2023) Microbiome and human health: current understanding, engineering, and enabling technologies. *Chem Rev* **123**, 31–72.
5. Maynard C & Weinkove D (2018) The gut microbiota and ageing. *Subcell Biochem* **90**, 351–371.
6. Markaki M & Tavernarakis N (2020) *Caenorhabditis elegans* as a model system for human diseases. *Curr Opin Biotechnol* **63**, 118–125.
7. Weinkove D & Zavagno G (2021) Applying *C. elegans* to the industrial drug discovery process to slow aging. *Front Aging* **2**, 740582.
8. Iyer S, Sam FS, DiPrimio N *et al.* (2019) Repurposing the aldose reductase inhibitor and diabetic neuropathy drug epalrestat for the congenital disorder of glycosylation PMM2-CDG. *Dis Model Mech* **12**, dmm040584.
9. Kenyon CJ (2010) The genetics of ageing. *Nat* **464**, 504–512.
10. Sándor S & Kubinyi E (2019) Genetic pathways of aging and their relevance in the dog as a natural model of human aging. *Front Genet* **10**, 948.
11. Revelas M, Thalamuthu A, Oldmeadow C *et al.* (2018) Review and meta-analysis of genetic polymorphisms associated with exceptional human longevity. *Mech Ageing Dev* **175**, 24–34.
12. Felix MA & Duveau F (2012) Population dynamics and habitat sharing of natural populations *of Caenorhabditis elegans* and *C. briggsae*. *BMC Biol* **10**, 59.
13. Weinkove D (2015) Model super-organisms: can the biochemical genetics of *E. coli* help us understand aging? *Biochem* **37**, 12.
14. Virk B, Correia G, Dixon DP *et al.* (2012) Excessive folate synthesis limits lifespan in the *C. elegans*: *E. coli* aging model. *BMC Biol* **10**, 67.
15. Virk B, Jia J, Maynard CA *et al.* (2016) Folate acts in *E. coli* to accelerate *C. elegans* aging independently of bacterial biosynthesis. *Cell Rep* **14**, 1611–1620.
16. Zavagno G, Raimundo A, Kirby A *et al.* (2024) Rapid measurement of ageing by automated monitoring of movement of *C. elegans* populations. *GeroScience* **46**, 2281–2293.
17. Fox JT & Stover PJ (2008) Folate-mediated one-carbon metabolism. *Vitam Horm* **79**, 1–44.
18. Magnúsdóttir S, Ravcheev D, de Crécy-Lagard V *et al.* (2015) Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front Genet* **6**, 148.
19. Halsted CH, Ling EH, Luthi-Carter R *et al.* (1998) Folylpoly-gamma-glutamate carboxypeptidase from pig jejunum. Molecular characterization and relation to glutamate carboxypeptidase II. *J Biol Chem* **273**, 20417–20424.
20. Hackmann C (1958) Observations on influenceability of age phenomena in experimental animals by peroral administration of combinations of 2-(p-aminobenzolsulfonamide)-pyrimidin. *Munch Med Wochenschr* **100**, 1814–1817.
21. Miazga A, Osiński M, Cichy W *et al.* (2015) Current views on the etiopathogenesis, clinical manifestation, diagnostics, treatment and correlation with other nosological entities of SIBO. *Adv Med Sci* **60**, 118–124.
22. Camilo E, Zimmerman J, Mason JB *et al.* (1996) Folate synthesized by bacteria in the human upper small intestine is assimilated by the host. *Gastroenterology* **110**, 991–998.
23. Stocke KS & Lamont RJ (2024) One-carbon metabolism and microbial pathogenicity. *Mol Oral Microbiol* **39**, 156–164.
24. Maynard C, Cummins I, Green J *et al.* (2018) A bacterial route for folic acid supplementation. *BMC Biol* **16**, 67.
25. Han B, Sivaramakrishnan P, Lin CJ *et al.* (2018) Microbial genetic composition tunes host longevity. *Cell* **173**, 1058.




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