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Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans

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It is often presented as common knowledge that, in the human body, bacteria outnumber human cells by a ratio of at least 10:1. Revisiting the question, we find that the ratio is much closer to 1:1.

The human microbiome has emerged as an area of utmost interest. The last two decades have produced an avalanche of studies revealing the impact that the microbiota have on the physiology and metabolism of multicellular organisms with implications for health and disease. One of the most fundamental and commonly cited figures in this growing field is the estimate that bacteria residing in the human body outnumber human cells by a factor of 10 or more (Figure 1A). This striking statement often serves as an entry point to the field. After all, if a human being is a cell population composed of at least 90% bacteria, it is only natural to expect a major role for them in human physiology.

Both the numerator (number of microbial cells) and the denominator (human cells) of this 10:1 ratio are based on crude assessments. Most sources cite the number of human cells as 10^{13} or 10^{14}, and a recent study reported 3.7×10^{13} human cells in a "reference" human (Bianconi et al., 2013). Estimates for the number of microbial cells in the body (which we operationally refer to as bacteria as they overwhelmingly outnumber eukaryotes and archaea in the human microbiome by 2 to 3 orders of magnitude) are usually 10^{14}–10^{15} (Berg, 1996; Savage, 1977). We performed a thorough review of the literature and found a long chain of citations originating from one "back of the envelope" estimate (Figure 1). This estimate, though illuminating, was never meant as the final word on the question.

Recently, the estimate of a 10:1 bacterial to human cell ratio (B/H) ratio has received criticism (Rosner, 2014). Therefore, an alternative value and an estimate of the uncertainty range are needed.

The Number of Bacteria in the Body

Bacteria are found in many parts of the human body primarily on the external and internal surfaces, including the gastrointestinal tracts, skin, saliva, oral mucosa, and conjunctiva. The vast majority of commensal bacteria reside in the colon, with previous estimates of about 10^{14} bacteria (Savage, 1977), followed by the skin, which is estimated to harbor $\sim10^{12}$ bacteria (Berg, 1996). Less than 10^{12} bacteria populate the rest of the body (Berg, 1996; Tannock, 1995). Within the alimentary tract, the colon is the dominant contributor to the total bacterial population, while the stomach and small intestine make negligible contributions. As a result, the colon is the focus for estimating the number of bacteria in the body. Almost all recent papers in the field of gut microbiota directly or indirectly rely on a single paper (Savage, 1977) discussing the overall number of bacteria in the gut. Interestingly, review of the original paper (Savage, 1977) demonstrates that it actually cites another paper for the estimate (Luckey, 1972). The citation lineage for a few representative cases, tracking back to the original calculation, is illustrated in Figure 1A. The progenitor paper performed an order-of-magnitude estimate by assuming 10^{11} bacteria per gram and 1 L (or about 1 kg) of alimentary tract capacity. The estimate, performed by Luckey in 1972, is an illuminating example of a back-of-the-envelope estimate, which was elegantly performed, yet was probably never meant to serve as the cornerstone reference number to be cited decades later. On top of this historical contingency, a recent report from the NIH stated that 1%–3% of body mass is composed of bacteria (with no reference ascribed; see Sender et al., 2016). This value, quoted in many online resources such as Wikipedia, coupled with a rule of thumb of 1 μm^3 for bacterial cell volume, suggests an estimate of 10^{15} bacteria in the human body, which led to claims of a B/H ratio of 100:1.

The estimate of the number of bacteria in the human colon (Luckey, 1972), which we identify as the primary reference across the literature, was made by taking the volume of the alimentary tract, assumed as 1 L, and multiplying it by the number density of bacteria, assumed to be 10^{11} bacteria per gram of wet content as shown in Figure 1B. However, the number of bacteria in the alimentary tract proximal to the colon is negligible in comparison to the colonic content, and thus the relevant volume for the 10^{11} bacteria/g density is only that of the colon. The inner volume of the colon in the reference adult male is estimated through various methods as 340 ml (Eve, 1966) or 480 ml (Pritchard et al., 2014).

A survey of 14 literature sources gives a mean value of 0.9×10^{11} bacteria/g wet stool (with an uncertainty of 19% and a coefficient of variation [CV] of 46%) (Sender et al., 2016). Assuming that wet stool is representative of the colon content and a colon content volume of 0.41 L, we have 3.9×10^{13} bacteria in the colon with an uncertainty of 24% and a variation of 52% over a population of standard weight males. Considering that the

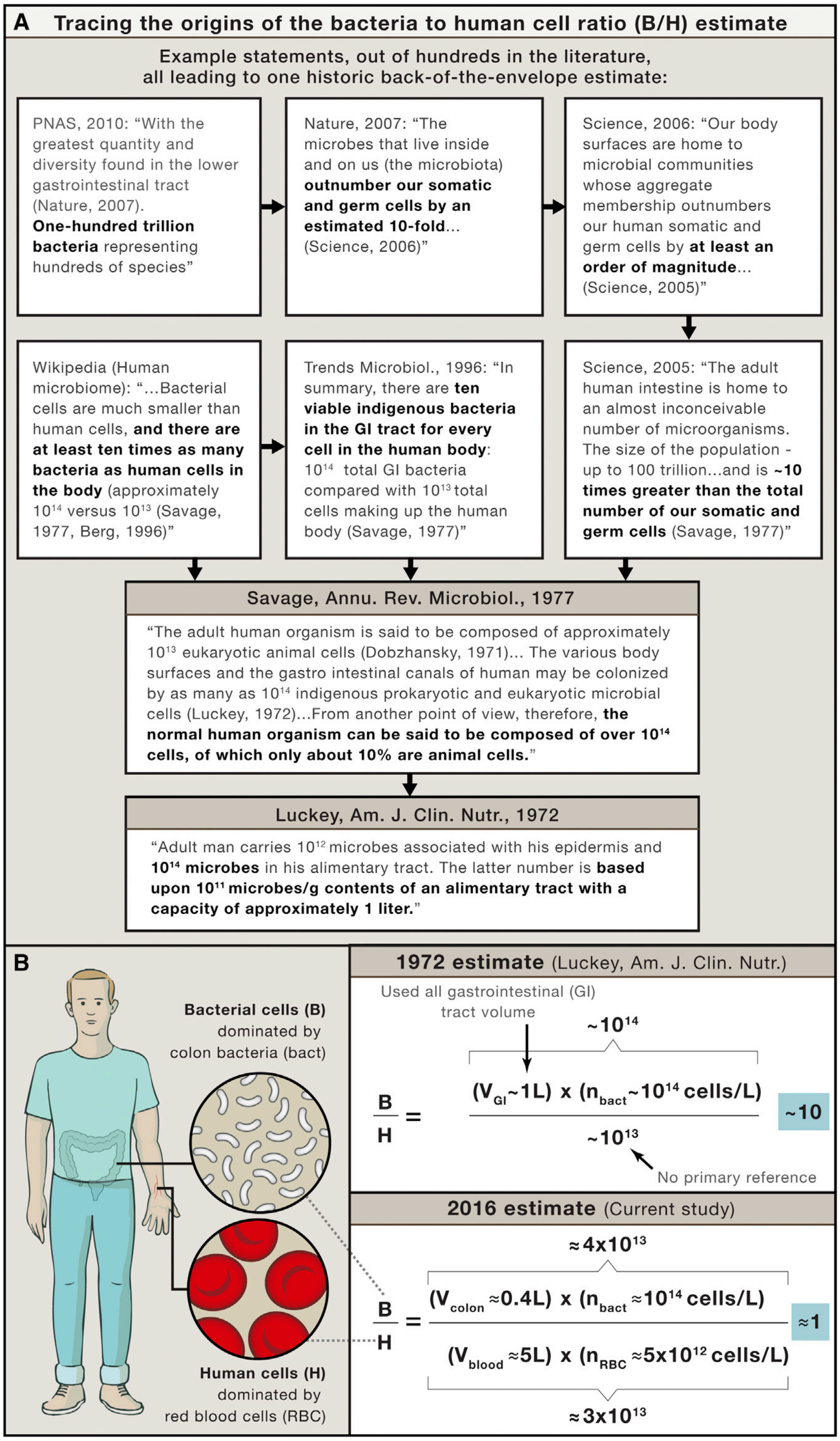

Figure 1. The Ratio of Bacteria to Human Cells

(A) A non-exhaustive lineage tree of quotations showing the origins of the often-quoted sources for the number of bacteria in the human gut. The 1977 review by Savage is referenced over 1,000 times in the literature, often in the context of the estimate for the vast overabundance of bacteria over human cells. Brief quotes from the original papers are shown. Arrows point to the reference used. The numerical statements are in bold. For detailed references see, Sender et al. (2016).

(B) Comparison between the well-cited estimate (Luckey, 1972) and the current estimate, highlighting the key four parameters identified as determining the B/H ratio in the standard man. Note that, in line with formal definitions, we use "$\sim$" to denote "order of magnitude" and "$\approx$" to denote "approximately equal" (usually to better than 2-fold).

contribution to the total number of bacteria from other organs is at most 10^{12}, we use 3.9×10^{13} as our estimate for the number of bacteria in the "reference man."

The Number of Human Cells in a "Standard" Adult Male

In the literature, we find many statements for the number of cells in the human body ranging from 10^{12} to 10^{14} cells. A mass-based order-of-magnitude estimate for this number assumes a 10^2 kg man, which is divided by the mass of a "representative" mammalian, cell 10^{-12}–10^{-11} kg (assuming cell volumes of 1,000–10,000 μm^3, respectively), thus arriving at 10^{13}–10^{14} cells.

A more nuanced approach that bypasses the need to think of a representative "average" cell systematically counts cells by type. A detailed analysis of this sort was recently published (Bianconi et al., 2013). The number of cells in the body by type or organ system was estimated. Since the aim was to systematically scrutinize all cellular components, the authors alternate between grouping by histologic type (e.g., glial cells) or by locus/organ where both parenchymal and stromal cells are accounted for (e.g., "bone marrow nucleated cells"), totaling 56 cell type categories. We revisited and updated the values for all the main contributors to total cell number to find 3.0×10^{13} human cells in the 70 kg "reference man" with 2% uncertainty and 14% CV (Sender et al., 2016).

The most ubiquitous contribution (84%) to the overall number of cells comes from red blood cells. The average blood volume of 4.9 L (SEM 1.6%, CV 9%), multiplied by a mean red blood cell count of 5.0×10^{12} cells/L (SEM 1.2%, CV 7%), leads to a total of 2.5×10^{13} red blood cells (SEM 2%, CV 12%). Other major contributors are platelets (5%), bone marrow cells (2.5%), lymphocytes (2%), and endothelial cells (2%) (Sender et al., 2016). One conspicuous observation from this detailed account is that over 90% of human cells originate from the hematopoietic stem cell. The striking dominance of the hematopoietic lineage in cell count is counterintuitive, given the composition of the body by mass, which is dominated by muscle and fat cells. This seeming discrepancy stems from the relatively small size of blood cells.

The Ratio of Bacteria to Human Cells in the Adult Body

After revising both the numerator and denominator in the ratio of bacteria to human cells in the body, we arrive at our updated estimate of B/H = 1.3, with an uncertainty of 25% and a variation of 53% over the population of standard 70 kg males. Comparison between the current estimate and the original estimate is illustrated in Figure 1B.

We think that this value and uncertainty are a much more realistic depiction that should replace the 10:1 or 100:1 values, which are common in the literature; at least until more accurate measurements become available.

Interestingly, if we compare the number of bacteria in the human body (3.9×10^{13}) to the number of nucleated human cells ($\approx 0.3 \times 10^{13}$) we do get a ratio of about 1 to 10. We note that this ratio is the result of both the number of bacteria and the number of nucleated human cells in the body to be several times lower than in the original estimate (that did not restrict the analysis to nucleated cells).

The standard person used in the literature and thus analyzed above is defined as a "reference man being between 20 and 30 years of age, weighing 70 kg, is 170 cm in height" (Snyder et al., 1975). We now discuss the updates required in the calculation and the applicability of our conclusions to other segments in the population. To explore the effect of factors such as age, gender, and body weight, we focus on the four parameters (Figure 1B), which dominate any quantitatively significant deviations from the standard reference. This is because the colon bacterial count and total RBC count dominate either side of the B/H ratio. The four parameters are, therefore, colon volume and bacterial density in the colon on the one hand and hematocrit and blood volume on the other. Let us start with the gender effect. Colon volume in females is similar to that of males, 430 ± 170 ml for a female of "standard" 1.63 m height (ICRP, 2002; Pritchard et al., 2014). As for colonic/fecal bacteria number density, there is no report in the literature of gender-specific differences. The number of red blood cells is affected by the total blood volume and by the red blood cell concentration. Red blood cell concentration is about 10% lower for females (Wakeman et al., 2007). Furthermore, blood volume is also lower by about 20%–30% (Boer, 1984). Therefore, we expect the bacteria to human cell ratio to increase by about a third in females.

Proceeding to analyze infants, we note that colon bacterial density is relatively constant from infancy to adulthood (Sender et al., 2016). Colon volumes for the pediatric population, reported as 50 ml for neonates and 80 ml for 1-year-old infants (ICRP, 2002), are derived only from comparing infant to adult daily fecal output values and are thus less reliable and represent a knowledge gap. RBC concentration in the blood has a characteristic small temporal variation from the neonate to the elderly. RBC count values at birth are somewhat higher than for normal adults but they decrease during the first 2 months until they level at 10% lower than adult values. On the other hand, the blood volume to weight of infants is 75–80 ml/kg, $\sim$10% higher than normal adults (Sender et al., 2016). Therefore, the overall effect in terms of RBC count per body mass is smaller than 10%. In the elderly, blood volume is reduced by about 25% (Davy and Seals, 1994), while the hematocrit is essentially unchanged. We therefore conclude that the effect of age on the B/H ratio is smaller than 2-fold from age 1 year onward and probably within the variation we estimated across the population of "standard" adult males.

Finally, we analyze the effect of obesity, which is of interest in our context considering the highly intriguing links between gut microbiome and weight. Measurements of the colonic bacterial concentrations in obese individuals are similar to the ones for the reference man (Sender et al., 2016), indicating that the change in total bacteria number as a function of weight is determined only by the change in colon volume. We could not find any direct measurements of the colonic volume for obese individuals in the literature, yet from an indirect analysis, the volume increases with weight and plateaus at about 600 ml, i.e., about 50% higher than that of the standard man value (Sender et al., 2016). Moving to the number of human cells, we note that the excess body weight in high BMI individuals is mostly contributed by adipocyte hypertrophy and hyperplasia. Since, in the reference man, fat tissue accounts for only 0.2% of the total human cell count (Sender et al., 2016), the added fat tissue accounts for a negligible contribution to the total human cell count. Blood volume itself increases with BMI. Because adipose tissue is not highly vascular, an increase of 100%–200% from the reference man's body weight to total body weights of 140–210 kg increases the total blood volume by 40%–80% (Feldschuh and Enson, 1977). This increase in blood volume is of the same range as the increase in colonic volume in the obese, and thus the B/H ratio is expected to remain within the uncertainty range we report for the "standard man." In conclusion, the paper's framework and general inferences on the B/H ratio are relevant for the general human population with minor quantitative differences.

We view this manuscript as a call to revitalize efforts in the direction of quantifying absolute cell content of human tissues and their commensal bacteria. Updating the ratio of bacteria to human cells from 10:1 or 100:1 to closer to 1:1 does not take away from the biological importance of the microbiota. Yet, we are convinced that a widely stated number should be based on the best available data, serving to keep the quantitative biological discourse rigorous. Investigating whether the concentration of bacteria in stool resembles that of the colon is an important avenue along which further study is required. The analysis presented here helps us achieve a more stable quantitative basis for discussing the cellular composition of the human body. Although we still appear to be outnumbered, we now know more reliably to what degree and can quantify our uncertainty about the ratios and absolute numbers. The B/H ratio is actually close enough to one, so that each defecation event, which excretes about 1/3 of the colonic bacterial content, may flip the ratio to favor human cells over bacteria. This anecdote serves to highlight that some variation in the ratio of bacterial to human cells occurs not only across individual humans but also over the course of the day. In addition, some medical procedures (e.g., bowel preparation before colonoscopy) decrease the bacterial colon content much more extremely than defecation and thus make the ratio significantly smaller than 1 for a period of hours to days.

In conclusion, we do not claim that a B/H of ~1 rather than ~10 should change the importance one gives to the subject of host-microbiota interactions. We do hope that our analysis will correct inaccurate quantitative statements and cause people to focus on more meaningful statements to explain the motivation for studying the microbiota. We think that the kind of progression presented in this study from informative back-of-the-envelope calculations to more nuanced value estimates is of wide interest and is instructive in the quantitative training of biologists. In performing these kinds of calculations, we become intimately familiar with the limits of our current understanding and, therefore, more easily highlight the best avenues for scientific progress in a particular field. What better place to start such quantitative training than by examining the contents of the human body? In doing so, we can comply with the Delphic maxim of "know thyself" in a truly quantitative fashion.

AUTHOR CONTRIBUTIONS

R.S., S.F., and R.M. conceived and performed the study and wrote the manuscript.

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