Nederbragt, Hubertus, Strategies to improve the reliability of a theory: the experiment of bacterial invasion into cultured epithelial cells, *Studies in History and Philosophy of Biological and Biomedical Sciences*, 34, 2003, 593-614.

Keywords

Robustness, experimental strategies.

Domain

Biology.

Abstract

The article presents a critical reconstruction of different strategies that have been used to justify the theory of bacterial invasion into cultured epithelial cells. It highlights that a single type of experiment often turns out to be insufficient to prove the reliability of a hypothesis, given the existence of alternative underdetermined theoretical interpretations both of the experimental procedures and of its results. The search of independent experimental support is shown to be the most widespread strategy to enhance the confidence in a hypothesis. The article characterizes multiple derivability as a particularly effective strategy that has to be distinguished both from reliable process reasoning and from variation of independent methods. Finally, the author develops an analysis of the role played by multiple derivability in anchoring a local theory in a larger theoretical network.

Development

The starting point of Nederbragt's analysis is the description of an experiment aimed at verifying the hypothesis of bacterial invasion into epithelial cells, hypothesis that the author qualifies as a *local theory*. The experimenters place a suspension of a known concentration of *Escherichia Coli* on an *in vitro* culture of monolayers of epithelial cells extracted from the udders of normal cows. The bacteria and the cells are then cultured at 37° C for one hour. Given that bacteria that do not remain free in the solution must either invade the cell or adhere to its external surface, the monolayers are washed to remove free bacteria and, then, the bacteria that have adhered to the cell without invading it are killed with antibiotics. The monolayers are then washed again to remove the antibiotics and the cells are lysed in order to count the bacteria that have actually invaded them. The counting indicates that a number of bacteria has actually invaded the cells and, therefore, is responsible for the infection. Now, is this the only way to interpret the outcome of the experiment? No. As a matter of fact, some of the extracellular bacteria that have adhered to a cell without invading it might have survived the action of the antibiotics and interfered with the final counting in a way that is hard to evaluate. Indeed this might happen as a result of the local production of antibiotic-degrading enzymes by the epithelial cell. Nederbragt concludes: "Thus the theory, based on colony count alone, is certainly underdetermined. The theory needs more support and it is here that multiple derivability comes in as a major strategy" (p. 596). Nederbragt stresses that repeating the experiment with an other antibiotics would not constitute a real independent test of the theory of bacterial invasion, for the new experiment would be ultimately based on the same theoretical background of the previous

one. An independent test would consist, for instance, in the observation of bacteria inside the cultured cell using electron microscopy, a method that is based on a theoretical background completely different from the biological knowledge underlying the counting of bacteria. Is multiple derivability the strategy actually followed by experimenters working in this specific field of research? In the third section of the article Nederbragt presents an extensive survey of the scientific literature on bacterial invasion from 1916 to 1982, which clearly indicates that the answer should be a positive one. "… most of the experimenters shown in the table used at least two methods to support their conclusions; examples are colony counting combined with photographs (…), radioactive bacteria combined with EM [electron microscopy] and light microscopy (…), phase contrast microscopy with fluorescent antibodies (…), and so on. Only a few consider one method sufficient" (p. 603). Nederbragt concludes that multiple independent derivation has been a widespread "type of reasoning" for proving the theory of bacterial invasion into cultured cells.

The example of bacterial invasion along with a reconstruction of the raisons why the hypothesis of the mesosome (a supposed cytoplasmatic organelle in bacteria whose existence seemed to be proved by observations carried out with electron microscopy) was abandoned suggests a hierarchical model of different types of reasoning allowing the development and confirmation of a local theory. Nederbragt suggests a hierarchy of three different methods of theory construction: 1) reliable process reasoning, 2) variation of independent methods, 3) multiple derivability. The first level consists in a thorough and systematic check of all the steps included in an experimental procedure whose reliability or application is being questioned. Systematic errors and possible artifacts are taken into account, but only minor modifications of the experimental protocols are applied at this stage. In the case of the bacterial invasion experiments, one might for instance modify "the cultivation times of mammary gland cells before the bacteria are added" (p. 609). The second strategy consists in small modifications of the experimental procedure that leave intact its theoretical background. At this level the experimenter can use gland cells from another cow. In the case of microscopy, an example of this type of variation is given by the adoption of a different fixation technique for the sample (e.g. fixation by freezing instead of fixation by chemicals). The third level consists in the implementation of two or more techniques that are based on different theoretical bodies ok knowledge. Clearly, the reliability of a theory increases with the subsequent application of each method. The hypothesis of the mesosome, for instance, was abandoned because it failed to pass tests at level 2) and 3).

It is noteworthy that Nederbragt does not equate multiple derivability with robustness, for, after presenting this hierarchy of strategies, he claims: "At each level, robustness is the result of a dynamical process of reasoning and theory making" (p. 609) and, already in the discussion of the example of the mesosome, we find the following statement: "Both, multiple derivability and variation of independent methods produce robustness but on different levels." (p. 606). Robustness is therefore intended as a generic property of reliability/solidity obtainable with different strategies among which the most effective is multiple derivability. The latter, in contrast with variation of independent methods, is also deemed capable of yielding more knowledge of the phenomena under study. This is Nederbragt's conclusion at least as far as the example of the mesosome is concerned: "Multiple

derivability may tell us something about the possible nature of our observations, variation of independent methods cannot do this" (p. 607).

The last section of the article is devoted to the notion of anchoring of a theory into a larger network. As multiple derivability consists in giving support to a theory on the basis of methods based on different bodies ok knowledge, its application enhances at the same time the anchoring of the theory in a vast network of scientific knowledge. Finally, Nederbragt adds that this anchoring as both a social and an historical dimension. Multiple derivable theories are bound to create a stronger consensus in the relevant scientific community, and, at least in the case of the study of bacterial invasion, the different techniques of derivation that were adopted showed a great continuity with the pre-existing tradition of research about phagocytosis.

Methodology

Conceptual analysis of ongoing scientific activities and of past controversies in the domain of cell biology. Extensive analysis of the relevant scientific literature.

Specific contributions

The article offers a clear analysis of multiple derivability and, thanks to a well documented historical survey, provides evidence for its widespread application in cell biology. Furthermore, the examples discussed suggest a conceptual demarcation of multiple derivability from other, less effective methods of improving the reliability of a theory.

Entry by Emiliano Trizio: emilianotrizio@hotmail.com