Series

What history tells us XV. Cyril Norman Hinshelwood (1897–1967) – A chemical dynamic vision of the organic world

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1. The ambiguous contributions of Hinshelwood to modern biology

Whereas the value of the work of Hinshelwood is well acknowledged in physical chemistry (Laidler 1993), his contributions to modern biology are more controversed. He is credited with having been the first to propose that the sequence of nucleotides is responsible for the determination of the sequence of aminoacids in proteins, in an article published in the *Journal of the Chemical Society* in 1950 (Caldwell and Hinshelwood 1950b): the polypeptide chain is progressively elongated on a polynucleotide matrix of RNA by a process similar to crystallization. A strong argument in favour of the existence of such a process was the similar value (3.4 Angstroms) separating nucleotides in an elongated chain and amino acids in proteins, revealed by the X-ray diffraction studies performed by William Astbury in Leeds (Astbury and Bell 1938). In addition, since the number of different amino acids in proteins (23 for Hinshelwood) was far more than the number of different nucleotides (5), Hinshelwood argued that the nucleotides at positions n-1 and n+1 ought to participate to the choice of the amino acids, which retrospectively can be seen as an anticipation of the triplet code. By proposing this new model for protein synthesis, Hinshelwood opposed the so far dominant model – the multi-enzyme model – in which a battery of proteases was in charge of the synthesis of proteins by operating in the reverse direction (Fruton 1941; Bartels 1983). Hinshelwood's proposal preceded the nucleic acid template model of Alexander Dounce (Dounce 1952, 1953), a model frequently considered as the first explicitation of the relations between nucleic acids and proteins, by two years. In addition, he opposed the multi-enzyme model for a reason different from that outlined by Dounce; whereas Dounce outlined the regression ad infinitum of the multi-enzyme model – multi-enzymes must be responsible for the synthesis of… multi-enzymes –, Hinshelwood showed the impossibility for this model to account for the specificity of protein sequences. The contribution of Hinshelwood was acknowledged by Francis Crick in 1966 (Crick 1966). Nevertheless, it remains difficult to demonstrate that his article was influential. George Gamow, who was the first to propose a genetic code, few months after the description of the double helix structure of DNA, did not mention Hinshelwood's hypothesis (Gamow 1954). And for Hinshelwood, the relation between protein and polynucleotide sequences was reciprocal, a protein being able to guide the synthesis of a polynucleotide, a possibility rejected as heretical by present-day biology. An additional merit of Hinshelwood, retrospectively not so small, was to receive Sydney Brenner when he arrived from South Africa, and to orient him towards the study of bacteriophage (Judson 1996).

In fact, Hinshelwood is also known as the one who tried to explain the phenomenon of adaptation of microorganisms to new nutrient sources or drugs by the spontaneous reequilibration of a complex set of biochemical reactions. He strongly opposed the idea that adaptation was the result of the selection of variants in the bacterial population. He saw adaptation as a heritable process resulting from the response of organisms to variations in their environment, a clear Lamarckian process. When he presented this vision of adaptation in 1946 in *The chemical kinetics of the bacterial cell* (Hinshelwood 1946), the complexity of data in this research field, the lack of a clear distinction between evolutionary adaptation in general and the specific process

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observed in microorganisms of adaptation to nutrient sources, made such an hypothesis tenable. Nevertheless, Salvador Luria and Max Delbrück, ignored in Hinshelwood's book, had already brought strong experimental arguments in favour of a Darwinian model of bacterial adaptation (resistance) to bacteriophages (Luria and Delbrück 1943). In 1966, after the description of the operon model explaining how bacteria adapt to a change in their nutrient sources, Hinshelwood published a second book *Growth, function and regulation in bacterial cells*, in which he persisted in considering adaptation as a simple, partially inheritable, kinetic reequilibration (Dean and Hinshelwood 1966; Müller Hill 1996). This book is frequently ommitted in the official bibliographies of Hinshelwood.

So the contributions of Hinshelwood present themselves as a combination of openness to new models and attachment to old ones. Probably more significant is the strong continuity between his pioneering work in physical chemistry and his contributions in biology. We will show that the coherence of Hinshelwood's work originates in his chemical vision of organismic phenomena.

2. From physical chemistry to biology

The career of Hinshelwood was brilliant. Fellow of the Royal Society at the age of 32, he was its president from 1955 to 1960. He spent all his scientific life in Oxford, where he was nominated Professor in 1937. In 1956, he received the Nobel prize in chemistry with the Soviet chemist Nikolay Semyonov for their work on chain reactions. His First World War experience on explosives was decisive for the rest of his career. He contributed to the description of unimolecular reactions – resulting in fact from the activation of molecules by their encounter with similar molecules – by underlining the importance of a geometrical factor making these interactions efficient. He is best known to have demonstrated that the formation of water from the combination of oxygen and hydrogen is a chain reaction. He showed that chain reactions can be branched, and underlined the preeminent place of radicals in these types of reactions. He also demonstrated the importance of surfaces in many chemical reactions. With the help of these different observations, he was able to explain why an explosion may occur when the size of the reaction chamber is changed, for instance from the laboratory to the factory, the so-called "scale effect": certain chemical processes are dependent upon the volume, whereas others are controlled by the surface of the vessel in which the reaction takes place; when the size is changed, their ratio does not remain constant. The work of Hinshelwood had practical consequences for everyday life: the charcteristics of gasoline and the synthesis of artificial polymers benefiited from his fundamental contributions. In addition, Hinshelwood was the author of fundamental books in physical chemistry, some of which have been recently reprinted (Hinshelwood 1926; 1951).

He turned to biology at the end of the 1930s, and adopted one particular experimental system: the study of the growth of bacterial cultures in well-defined conditions. At the time it was widely used as a simple system to characterize substances essential for growth. The role (and nature) of many vitamins was determined thanks to such a system during these years. A bacterial growth curve is very simple, and can be decomposed into three phases: a lag phase, an exponential phase of growth – called the logarithmic or log phase –, and a third phase during which growth rate progressively decreases and the number of bacteria reaches a plateau. These curves, and in particular the log phase, were highly reproducible, and could be easily fitted by simple equations. The experimenter could easily play with such a system, changing the experimental conditions, pH, temperature, or adding new nutrient sources and drugs. He could look for changes occurring in the different phases of the growth process, i.e. during the adaptation of the organisms to the new conditions, and the transmission of these modifications in successive cultures. This experimental system, easy to manipulate by a non-biologist, was capable of generating a highly rich set of data.

For Hinshelwood, it simply was a prolongation of his early work. Metabolism is a complex ensemble of branched chain reactions. He did suspect, without strong arguments, that many of these reactions were producing radicals. He interpreted the phenomena of adaptation to drugs and nutrients as a re-equilibration, a restoration of the steady state with minor adjustments, an alternative mode of growth, eventually a change in metabolic routes. Relatively simple mathematical models, in which enzymes were considered as catalytic surfaces, could account for the changes in the shapes of growth curves which were observed during these treatments. The characterization and measurement of the activities of the enzymes involved in these processes could complement previous studies. To explain the adaptation to drugs – a process called "immunity" – was to explain the phenomenon of resistance (for instance to sulphonamides [Davies and Hinshelwood 1943] and antibiotics), and a way to overcome it. This work was therefore considered important from a medical point of view.

Hinshelwood performed many different studies from the end of the 1930s to the 1950s, which were published in a series of articles (see, for instance, Davies *et al.* 1944; Hinshelwood and Lewis 1947), and many were summarized in the 1946 book.

To explain the growth of the bacterial mass, Hinshelwood postulated that when an enzyme works, it leads, in addition to the formation of the product, to its own expansion according to the reaction

'Enzyme + substrate generates expanded enzyme + product'

After the second World War, he endeavoured to put some flesh on this process of self-synthesis. He was rapidly convinced of the importance of nucleic acids and RNAs in the synthesis of proteins, and DNA as a controller of cell division. He did experiments to demonstrate the distinct behaviour of these two nucleic macromolecules in different growth conditions (Caldwell and Hinshelwood 1950a).

His choice of bacteria as a model system was deliberate, and not uniquely due to their simplicity. He considered that the condensation of genes in chromosomes observed in higher organisms had isolated the genetic material from the environment, and made the rules of genetics dominate over the rules of chemistry. In contrast, the genetic material was dispersed in bacteria, "open" to modifications coming from the environment, and its properties were fully explainable by the laws of chemistry. He did not exclude the existence of mutations, but, in bacteria, limited their role to the generation of important variations. He considered that (a) the fact that bacteria were able to rapidly adapt to (nearly) any new drug and nutrient source to which they were put in contact, (b) the existence of training effects, i.e. that a progressive increase in a drug led to a progressively higher resistance to it, and (c) the delayed stabilization of the adapted state, were observations difficult to explain by the selection of variants in the bacterial population, but they were in full agreement with his own kinetic models (Davies *et al* 1945).

3. A fully coherent view of life

According to Hinshelwood, organisms can be envisaged as complex systems of chemical reactions, and there is no true discontinuity or boundary between the inanimate and the animate world; hence the possibility for a physical chemistry-based approach to explain the behaviour of simple organisms like bacteria. Hinshelwood was not original in adopting such a programme. As many others, he believed in the existence of a scale of beings, in which organisms can be positioned according to their degree of complexity. Bacteria, as organisms considered to be devoid of any structural organization, immediately above the threshold of life, were at the lowest position. He also shared the ambition of many chemists and physicists in those years to "naturalize" organisms and biological phenomena by abolishing the boundary between non-life and life. For Hinshelwood, death was nothing but the impossibility for a chemical sysyem to return to its steady state on account of the absence of some essential chemical intermediate. In the last chapter of *The structure of physical chemistry* entitled "The organic world" (Hinshelwood 1951), one of the paragraphs is titled "Growth and form", a clear reference to the highly influential book of D'Arcy Thompson, *On growth and form* (D'Arcy Thompson 1917). The ambition of Hinshelwood was similar to that of D'Arcy Thompson, which was to show that organisms can be explained by the laws of physics. But whereas D'Arcy Thompson concentrated his efforts on the macroscopic form of organisms, Hinshelwood studied their microscopic properties. He did not deny the existence of a genetic material in bacteria. But he considered that its macromolecular architecture remained simple: this material was not protected against the changes occurring in the environment, as it was in higher organisms. The consequence was that most of the heritable changes observed in microorganisms resulted from the direct effects of the environment on this material, and more generaly on the interlinking of the various chemical reactions occurring within them. Hinshelwood adopted a Lamarckian view of these organisms: the heredity of acquired characters is the dominant form of heredity. This was not the case in higher organisms, where the organization and isolation of the genetic material, as well as the general organization of organisms with the creation of a "milieu intérieur" made them insensitive to variations in the external environment.

The conceptions of Hinshelwood are very close from those of French neo-Lamarckians of the first decades of the 20th century. He shares with them the idea that plasticity and the power to adapt constitute fundamental characteristics of organisms. This neo-Lamarckian movement found strong support in the existence of the well-studied process of attenuation of microorganisms used for the production of vaccines during the first decades of the 20th century. As Laurent Loison has recently shown, the main difference between Darwinians and neo-Lamarckians was that, for the former, variations were the causes of adaptation, whereas for the latter they were mere effects of this adaptation (Loison 2008). For neo-Lamarckians, to hypothesize the existence of variations allowing the adaptation to any new growth condition was not a satisfactory explanation of what happened. It said nothing about the chain of events running between the changes occuring in the external medium and the new resulting properties of microorganisms. For Hinshelwood, only mechanistic explanations were valuable, and Darwinian ones were not. To the populational view supported by Darwinians, he opposed the conception that adaptation was an intrinsic property of every bacterium.

Organisms were conceived by Hinshelwood as chemical systems, susceptible in response to changes in the environment to shift from one stable state of functioning to a new one, the changed state being faithfully transmitted to subsequent generations. This conception is very similar to the model proposed by Max Delbrück in 1949 to explain how stable states could be transmitted through bacterial division (Delbrück 1949). The model of Delbrück is considered as one of the first models of epigenetic inheritance, forgotten when Jacques Monod and François Jacob proposed their

regulatory models for gene expression (Thieffry 1996), but recently rediscovered with the rise of systems biology, and the renewed interest for epigenetics. By these two characteristics, the models of Hinshelwood can be seen as an anticipation of present studies (see for instance the article by R Jayaraman [2008] on bacterial persistence in this issue). The approach of Hinshelwood was reductionist in the sense that biological facts were reduced to chemical ones. Simultaneously, it was not reductionist because the properties of the system emerged from a complex set of interwoven chemical reactions.

The evolutionary model of Hinshelwood was coherent with his functional conceptions: the autosynthetic capacities of organisms, associated with the existence of a genetic material, became independent of the environment only progressively; and in higher organisms, no longer obeyed chemical laws, but the mathematical laws of genetics. Our present vision is very different: the laws of biology and genetics concern all organisms, including microorganisms. The transition between chemical and biological laws took place earlier, during the prebiotic steps. If the models of Hinshelwood retain any value, it is to explain what happened in the prebiotic world.

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