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The quantitative analysis of drug–receptor interactions: a short history

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Pharmacology started to develop into a real quantitative science in 1909, when A.V. Hill derived the Langmuir equation in the course of his studies on nicotine and curare. A history of the developments since then shows both brilliant insights and missed opportunities. It also shows that much remains to be done. There is still no mathematical description that can describe quantitatively the actions of agonists on G-protein-coupled receptors, although progress has been greater with agonist-activated ion channels, which are much simpler.

Physics, mathematics and receptors

The origins of pharmacology lie in therapeutics, as shown by its early history (e.g. [1]). But receptors are protein 'machines' and it was not long before attempts were made to describe receptor properties using the laws of physics. This needed a knowledge of physics, and of the mathematical methods used by physicists, which were uncommon among pharmacologists. It was not until A.V. Hill [2], a mathematician turned physiologist, took an interest in drug receptors in 1909 that this process got started.

In this article, I shall describe the evolution of attempts to describe the physics of receptors in terms of equations. Sometimes this area is referred to as 'receptor theory' but this term covers much that is closer to empirical description than to physics, and therefore is not relevant to this discussion. The term 'receptor theory' therefore disguises an important distinction and I prefer not to use it. The number of key players in this drama is quite small (Figure 1), and naturally this narrow scope excludes many other important contributions to pharmacology. The developments described here transformed pharmacology from being descriptive therapeutics into being a fully-fledged science.

Archibald Vivian Hill (1886–1977)

The first really quantitative attempt to understand the relationship between drug concentration and response came from studies in Cambridge, UK [2]. A.V. Hill was Scholar of Trinity College, Cambridge in 1909 when he derived the expression that is often referred to as the Langmuir equation, although Langmuir's work came several years later [3,4]. The title page of the paper that, arguably, started quantitative pharmacology is shown in Figure 2.

Hill became an undergraduate at Trinity College in 1905 where he began studying mathematics. He won the college mathematical prize in 1906 and was Third Wrangler (ranked third among the first class) in Part I of the Mathematical Tripos in 1907. But, then he switched to natural sciences and spent two more years in physiology, chemistry and physics to complete Part II of the Natural Sciences Tripos in 1909, the year in which his first famous paper was published. The intellectual environment at Cambridge was astonishing. He attended lectures by G.H. Hardy and Alfred North Whitehead. His time at Trinity College overlapped with John Maynard Keynes (who became Hill's brother-in-law in 1913) and Bertrand Russell. He also encountered the classicist and poet A.E. Housman, who had returned to Cambridge in 1911 after having been Professor of Latin at University College London (UCL) since 1892 ([5,6]; http://www.physiol.ucl.ac.uk/Bernard_Katz/davidkatz.htm).

Hill started his physiological work under the supervision of J.N. Langley, with the help of a George Henry Lewes studentship from the Physiological Society. Lewes, incidentally, was a remarkable polymath, a founder member of the Physiological Society and partner of Marion Evans, better known as George Eliot, who founded the studentship in his memory; Lewes was credited with much of the medical input into that greatest of all Victorian novels, *Middlemarch* [7].

At Langley's suggestion, Hill's first project was to investigate the effects of nicotine and curare on contractures of the frog *rectus abdominis* muscle. He measured the time-course of responses and the relationship between nicotine concentration and the equilibrium response. He found that the time-course was exponential and said, "Two possible solutions can be advanced. The form of the curve is due either (a) to gradual diffusion of the nicotine inwards, or (b) to a gradual combination (chemical or otherwise) between nicotine and some substance in the muscle", the substance being, presumably, Langley's 'receptive substance'. In the course of testing the latter hypothesis he derived what later became know as Langmuir's adsorption isotherm (both the kinetic and the equilibrium forms), and showed that it described quite well both the rate and the extent of the action of nicotine.

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Archibald Vivian Hill (1886–1977, Cambridge and UCL). Hill (1909) discovered the Langmuir binding equation [9 years before Langmuir (1918)], and applied it to his studies on nicotine and curare.

Jeffries Wyman (1901–1995) (UCL, Harvard and Rome). The seminal article of Wyman and Allen (1951) [35] described how selective affinity for an active state was linked to conformation change. This was written in the context of haemoglobin (and enzymes). If it had been read by pharmacologists at the time it might have saved us a lot of argument and misunderstanding.

Alfred Joseph Clark (1885–1941, UCL and Edinburgh). Clark made the first serious attempts after Hill to apply physical laws to receptors. His book and reviews were very influential, although his analysis of competitive antagonism failed to identify the advantages of the dose-ratio approach.

Robert Stephenson (1925–2004, Edinburgh). Stephenson's influential 1956 paper proposed clearly that to understand an agonist it was important to distinguish between its ability to bind and its ability to activate once bound. He made a brave attempt to provide a general theory for agonists, based on the sort of null methods that Schild had exploited so successfully for antagonists. Sadly this proved over-ambitious (it is a pity that he was not aware of Wyman's work).

John Henry Gaddum (1900–1965, UCL and Edinburgh). Gaddum was the first to write the equation for competitive binding at receptors (in 1937, a Physiological Society abstract). But it referred to binding not response, and so was not usable until Schild's work. In fact, these equations date back to 1914, and appeared in Haldane's book *Enzymes*, published in 1930 [68].

Bernard Katz (1911–2003, UCL). In 1957, del Castillo and Katz, characteristically, proposed not a general theory but a very simple physical mechanism, in an attempt to explain the supposed partial agonist action of decamethonium. This mechanism was sufficient to illustrate beautifully the nature of the affinity–efficacy (or binding–gating) problem. It provided a counter example that showed that the Stephenson approach was wrong (although Wyman's work had actually already shown that in a much more general way).

Heinz Otto Schild (1906–1984, UCL). Schild showed, in 1949 and the 1950s, how to obtain the real equilibrium constant for an antagonist from measurements of responses, and so crude measurements such as IC_{50} values were no longer needed. This was enormously important because it was the first usable way of obtaining real physical information about receptors.

Alan Geoffrey Hawkes (1938–present, UCL, Durham and Swansea). Hawkes is responsible for much of the general theory underlying the interpretation of single-channel recordings. His work, in conjunction with the development by Neher and Sakmann of the patch-clamp method (1976), enabled the first separate measurements of affinity and efficacy (for the nicotinic acetylcholine receptor [52,72]).

Figure 1. Some people who have developed mathematical descriptions of the physics of receptors [32,52,68,72].

Hill cites Arrhenius's book *Immunochemistry* (published in 1907) [8] as authority for applying the law of mass action to a heterogeneous reaction at a surface. Unfortunately, he then went on to accept, on the basis of temperature dependence, the hypothesis that the time-course of his responses was limited by receptor interaction rather than diffusion (the misinterpretation of temperature dependence continued for decades and so it is hardly surprising that it was missed at this early stage). Nicotine and curare are described as 'antagonistic' drugs and Hill writes, "Prof. Langley has given reasons for supposing that curare, like nicotine, combines with the 'receptive substance' of muscles." Having got so very close, it seems odd, with the wisdom of hindsight, that Hill did not extend his analysis to the action of competitive antagonists, but that had to wait for John Henry Gaddum, 28 years later [well, actually, it was 4 years later (Box 1)].

The next year (1910), Hill [9] published what is now known as the Hill equation (the Hill plot came later [10]). This arose not through his study of nicotinic acetylcholine receptors but through a diversion to work with Joseph Barcroft on the interpretation of the latter's measurements of oxygen binding to haemoglobin. Hill was well aware of the lack of a physical basis for his derivation ("My object was rather to see whether an equation *of this type* can satisfy all the observations, than to base any direct physical meaning on n and K."). His caveat has often been forgotten since then.

Hill's achievement truly marks the beginning of quantitative pharmacology in addition to the emergence of biophysics as a distinct discipline. Although Hill soon left the receptor business to pursue the work for which he is more famous, muscle energetics, he also worked on haemoglobin, a molecule that has provided important insights into the workings of receptors (see later). The entry into biology of people like Hill, with a background in mathematics or physics, also emancipated these subjects from dominance by clinical medicine.

Hill left Cambridge in 1920, when he took the Brackenbury Chair of Physiology in Manchester, UK in 1920. In 1922 he won the Nobel prize, jointly with Otto Meyerhof, for his work on muscle physiology. In 1923 Ernest H. Starling was instrumental in getting Hill to come to UCL, where he stayed until his retirement in 1951. Initially, Hill held the Jodrell Chair of Physiology but from 1926 onwards he moved to the Royal Society Foulerton Chair. For most of that time, Hill headed a Biophysics unit, embedded in the Physiology Department.

THE MODE OF ACTION OF NICOTINE AND CURARI, DETERMINED BY THE FORM OF THE CONTRACTION CURVE AND THE METHOD OF TEMPERATURE COEFFICIENTS. By A. V. HILL, B.A., *Scholar of Trinity College, Cambridge.*

(*From the Physiological Laboratory, Cambridge.*)

In recent years there has been a tendency to attribute to a physical rather than to a chemical process, the action of many substances which have an effect upon the organism when given in very minute quantities. In very few cases, however, has the physical view been worked out in any detail. The actions of nicotine and of curari have been investigated by Prof. Langley on the lines of physiological experiment, and he advocates the view that these, and other similar bodies, in producing their specific effects form reversible chemical combinations with certain constituents—"receptive substances"—of the cells. In the following pages I have tested the mode of action of nicotine and curari, on the skeletal muscles of the frog, by mathematical and physico-chemical methods.

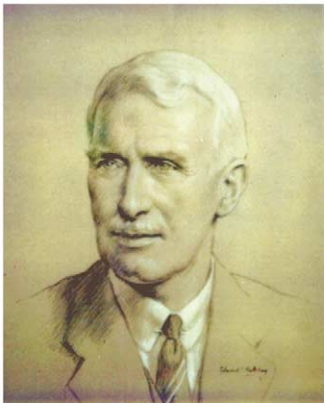


Figure 2. **(a)** The start of A.V. Hill's paper published in 1909 [2]. **(b)** Hill outside the University College London Physiology Department in 1923, chaired by students after he received a Nobel prize. **(c)** Hill in 1935 (drawn by Edward Halliday in 1978, from a photograph).

During his time at UCL he did more than superb science. In the 1930s, he was very active in the Academic Assistance Council, an organization that helped Jewish academics persecuted by the Nazi regime. From 1940 to 1945 he was a member of parliament (in those undemocratic times universities could nominate members). It is in no small part his doing that, when I arrived at UCL in 1964, two of UCL's most eminent professors, Bernard Katz and Heinz Schild (see later), had both been recruited after fleeing from Hitler.

Alfred Joseph Clark (1885–1941)

A.J. Clark was almost the same age as Hill and they graduated in the same year (1909). Clark succeeded Arthur Cushny in the Chair of Pharmacology at UCL in 1919, and stayed there for seven years before moving to Edinburgh in 1926 [11]. Clark was medical by background and his early papers did not show his interests in quantitative pharmacology (his first paper was, like Hill's, published in 1909 but its title was 'The detection of blood pigment in the faeces').

Clark's interests in quantitative pharmacology emerged towards the end of his time at UCL. In his papers on the actions of acetylcholine on frog *rectus abdominis* muscle and frog heart in 1926 he invokes the 'Langmuir adsorption equation', although strangely enough he does not attribute it to Hill, despite the fact that they overlapped at UCL for three years (one wonders if they ever met). Clark's book (published in 1933), *The Mode of Action of Drugs on Cells* [12], was a classic attempt to treat pharmacological problems using the methods of physical chemistry. The chapter titles would not be out of place today. Chapters 1 and 2 ('*The living cell considered as a physicochemical system*', and '*The fixation of drugs by cells*') establish much that we now take for granted: that drugs mostly work on the outside of cells and that only a small fraction of the surface of the cell needs to bind drugs to produce a response. Chapter 4, '*Physico-chemical laws applicable to drug receptor interactions*', shows a thoroughly modern appreciation of the use of the law of mass action (again attributed to Langmuir, not Hill) and of the problems caused by diffusion. In Chapter 5, '*The kinetics of cell–drug reactions*', Hill's 1909 paper is cited but only in connection with the empirical observation of exponential onset and offset. Yet the theoretical treatment of the concentration–response curve does not get far beyond the treatments in Hill's 1909 and 1910 papers. Clark, like Hill, speculated that the roughly hyperbolic shape of the curves might reflect 'Langmuirean' binding of agonists to receptors, although he was aware that this made assumptions that were impossible to justify.

Box 1. Competitive inhibition: the history

It seems odd, with the wisdom of hindsight, that it took until 1937 for the equation for competitive binding (see main text) to become widely known to pharmacologists. Exactly the same equation had been derived in 1928, 1930 and 1931 for the case of two gases competing for surface binding [65–67] and also, apparently independently, for competitive enzyme inhibition. Gaddum's equations appear explicitly in J.B.S. Haldane's book *Enzymes* (pp. 46–47), which was published in 1930 [68]. Haldane (Figure I) comments: "Hence the net effect of a competing substance is to increase K_m, and the amount needed to double it is equal to the dissociation constant [of the antagonist] K_f." This is essentially the same thing as Schild's pA_2. Haldane cites Michaelis, who seems to have been the first to derive the equation, which appears explicitly in two papers written in 1914 [69,70]. In fact something very close to it was already present in the original 1913 paper by Michaelis and Menten ([71]; http://web.lemoyne.edu/~giunta/menten.html) because this paper analyses the action of invertase on the assumption that all three ligands (the products glucose and fructose, and the substrate sucrose) compete for the same site. Maud Leonora Menten, incidentally, was one of the first Canadian women to get a medical degree (in 1911), but she moved to Berlin in 1912, where she got her Ph.D. in 1916 with Michaelis.

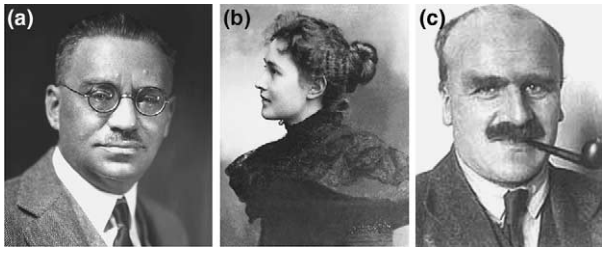


Figure I. Photographs of **(a)** Leonor Michaelis, **(b)** Maud Leonora Menten and **(c)** J.B.S. Haldane.

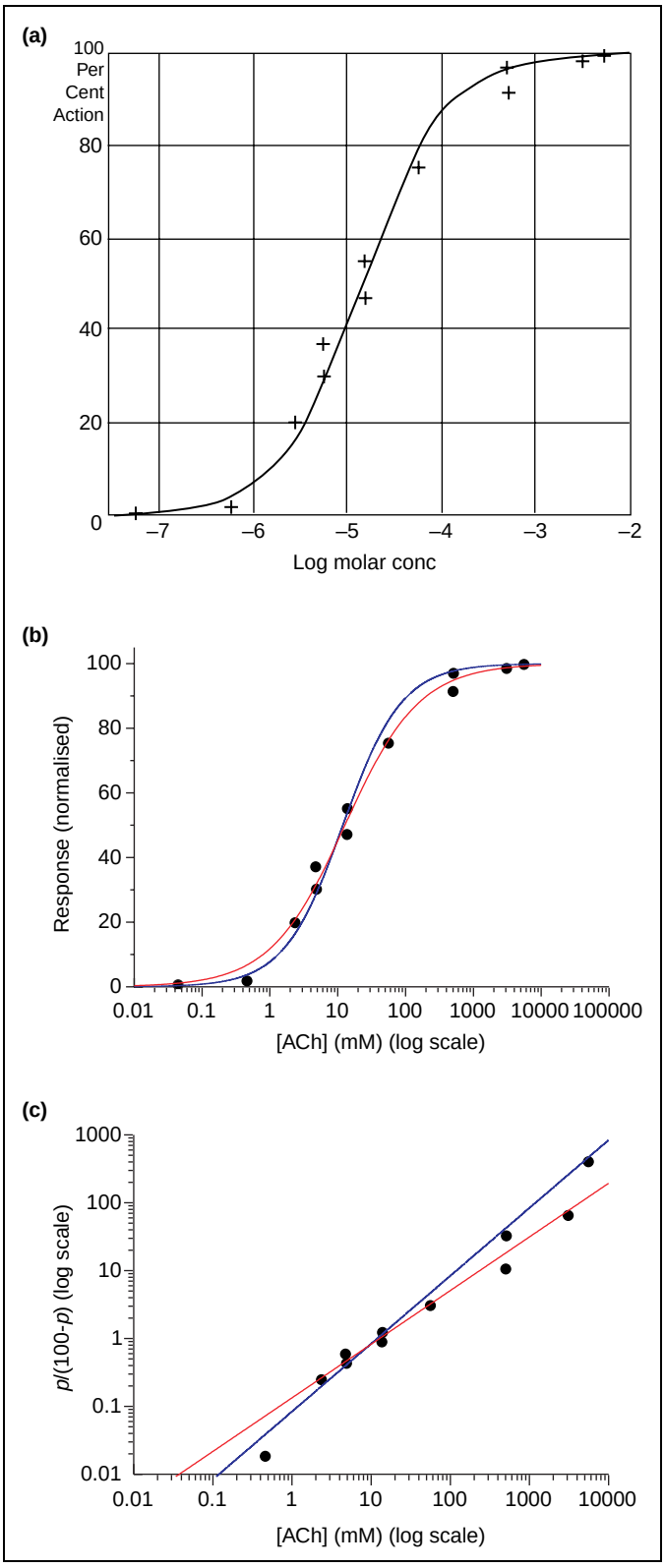


Figure 3. **(a)** Concentration–response curve for acetylcholine on frog *rectus abdominis* muscle, from A.J. Clark's paper published in 1926 [13]. He concluded that a Langmuir curve was a good description. **(b)** Re-plot of Clark's data with least-squares fit of Langmuir (blue) and Hill (red) equations; $n_H = 0.79 \pm 0.055$. **(c)** The same fits using Hill coordinates; note that extreme points should have lower weight (D. Colquhoun, unpublished).

It is interesting that if least-squares-curve fitting had been easier to implement in 1926, speculation might have been inhibited. Figure 3a shows data from Clark's first paper published in 1926 [13]. In Figure 3b his data are fitted with a Hill equation (red) and a Langmuir equation (blue). The Hill equation gives a Hill slope of $n_H = 0.79 \pm 0.055$, which is clearly less than $n_H = 1$ as in the Langmuir fit. Figure 3c shows why this is the case. The same data are shown as a Hill plot, and it is clear that the Langmuir fit gives too much weight to the most imprecise points: those near zero and 100%. It is interesting that Clark [14] starts by plotting his concentration–response curves as Hill plots (although again with no acknowledgement or explanation). The concept of the dose ratio was still missing, and Clark tried to relate antagonism to the relative concentrations of agonist and antagonist, although he notes that this was only an approximation. Clark concludes that very little acetylcholine is needed to produce effects (he estimates 20 000 molecules per heart cell [13]), but he also concludes in 1926, wrongly, that: "atropine and acetylcholine, therefore, appear to be attached to different receptors in the heart cells" [14]). Part of his reason for this wrong conclusion was that adding an excess of acetylcholine did not accelerate recovery from atropine block. He did not seem to be aware that the law of mass action predicts precisely this outcome (at least in the absence of diffusion barriers), because the time constant for dissociation of atropine, after the free concentration is reduced to zero, should be independent of the presence of other competing ligands, a fact that had been known to enzymologists since 1913 (Box 1).

When Clark revisited the question of antagonism some time later [15] he was much closer to the mark. In his article published in 1937 [15], Clark made the important observation that agonists of very different potency were antagonised to very similar extents by atropine. Furthermore, his article hints, parenthetically, at the best approach: "An alternative method of estimating the antagonistic power is to determine the concentration of B [antagonist], which alters by a selected proportion (e.g. tenfold) the concentration of A [agonist] needed to produce a selected effect" (p. 377). This is probably the first suggestion of the dose-ratio approach (see later) but it was not followed up. Despite the fact that this paper refers to the correct theoretical treatment of competitive antagonism, which had been published in the same year by Gaddum [16], it still fails to spot that the equation predicts that the dose ratio should be constant, rather than the ratio of agonist to antagonist concentrations. Nevertheless, the conclusion drawn was quite accurate: 'A considerable proportion of the facts observed can be interpreted on the assumption that antagonistic drugs compete for the same receptor.'

Clark had, incidentally, a side interest in debunking quackery. His papers in the British Medical Journal [17,18] are as relevant now as the day they were written. In the 1927 paper he discusses why even educated people may embrace quackery: "most modern Europeans would be either amused or disgusted by the Black Mass that was popular in the seventeenth century. To-day some travesty of physical science appears to be the most popular form of incantation". The only comment I can make on that is '*plus ça change plus c'est la même chose*' (see also http://www.ucl.ac.uk/Pharmacology/dc-bits/quack.html).

John Henry Gaddum (1900–1965)

Gaddum, like Clark, started in medicine (at UCL in 1922), and went on to work for Henry Dale [19]. Gaddum, like Schild, worked on the statistics of biological assays but his claim to fame in the limited area dealt with here is his short communication to the Physiological Society in 1937 [16]. This was the first time that the equation for the competitive binding of two ligands to a receptor was written explicitly. For two competing ligands, A and B, the receptor occupancy by drug A can be written simply in the form:

$$p_A = \frac{c_A}{1 + c_A + c_B},$$

where c_A is the normalised concentration of A, $c_A = [A]/K_A$, with K_A being the equilibrium dissociation constant for A and, similarly, $c_B = [B]/K_B$.

The description referred only to binding, not to response, and so it was not immediately useful for the analysis of experimental measurements of the effects of antagonists on the response to agonists. Putting it into a useful form for that purpose was the responsibility of Schild. In fact, this result was known long before 1937 (Box 1).

Heinz Otto Schild (1906–1984)

Schild was born in Fiume (now Rijeka, in Croatia), at a time when it was part of the Austro-Hungarian empire. In 1921, after the collapse of the Habsburg monarchy, his family moved to Munich, where he eventually graduated in medicine. In 1932, he moved to Dale's laboratory in London [20], and decided to stay in England when the Nazis came to power in 1933. After a period in Edinburgh, where he overlapped with Clark, Schild moved to UCL in 1937, where he stayed for the rest of his life, apart from a period of internment on the Isle of Wight between 1939 and 1940 (he was still an Italian citizen at the outbreak of war). Schild was the first of the people discussed here who I knew personally. He was the kindest of men (and he gave me my first job, as an assistant lecturer at UCL).

All the work described up to this point suffered from one enormous drawback: the equations all referred to binding. But, at this time, binding could not be measured directly with any precision, and the relationship between binding and response was unknown. Hill, Clark and Gaddum were aware of this problem but could not solve it. It was Schild's great achievement to make it possible, for the first time, to measure a physical quantity, the equilibrium constant for the binding of an antagonist, using simple measurements of the response of isolated tissues [21–24]. Schild generously attributed the idea to Clark and Raventos [15] and he also referred to the earlier work on competitive adsorption of gases (Box 1).

The key to circumventing the lack of knowledge about the relationship between agonist binding and response was to keep the response constant (a so-called null method). This trick meant that it had to be assumed only that occupancy of a specified fraction of receptors by agonist would always produce the same response, regardless of whether other receptors were occupied by antagonist. This was a far weaker assumption than had been made in earlier studies and it worked. The idea was to measure not the depression by antagonist of the response to a fixed concentration of agonist, but rather to measure the factor by which the agonist concentration had to be increased to keep the response the same in the presence of antagonist. This factor was known as the 'dose ratio'. Schild's name is now immortalised in the Schild equation, which gives the dose ratio r as:

$$r = 1 + \frac{[B]}{K_B},$$

where [B] is the antagonist concentration and K_B is the equilibrium dissociation constant for binding of the antagonist to the receptor. The Schild equation is often used in the form $\log(r-1) = -\log(K_B) + \log([B])$. The beautiful thing about this equation is that it does not refer to the agonist at all. It predicts that the equilibrium log(agonist concentration)–response curves will be shifted in a parallel fashion to the right (because r is a constant) to an extent that is independent of the nature of the agonist, and that a real physical quantity, K_B, can be estimated by measuring such shifts. This made Schild's approach infinitely preferable to measurements of IC_{50} values for antagonists, although sadly the latter are still common. Although Schild's original derivation was simple, it was subsequently shown that the Schild equation holds for a wide class of more complicated mechanisms [25]. It also holds for G-protein-coupled receptors (for which explicit formulation of agonist action is still impossible), as judged by many subsequent comparisons with direct binding measurements for antagonists. It really works. Of course, the Schild equation gives only an equilibrium constant. It has proved surprisingly difficult to find the rate constants for association and dissociation of competitive antagonists. For tubocurarine, for example, many attempts have been made to determine the rates (starting with Hill in 1909) but they all failed until 2001 [26].

The problem of extracting similar physical quantities for agonists, rather than antagonists, proved much more difficult and still remains unsolved for most receptors. Most of the subsequent work discussed here is concerned with this problem. The mathematical treatment of agonist action in the period 1950–1970 involved three main people, Robert P. Stephenson, Katz and Jeffries Wyman. Only Stephenson was a pharmacologist, and Wyman was not even talking about receptors, although arguably his contribution was the most far-sighted.

Robert Stephenson (1925–2004)

Stephenson worked for most of his life in the Pharmacology Department in Edinburgh. In 1956 he published a paper [27] that attempted to make sense of the recently discovered phenomenon of partial agonism [28]. Stephenson's paper was prescient: he pointed out that, although a binding constant was sufficient to characterize an antagonist at equilibrium, it was not enough for an agonist. For an agonist, one needed to consider not only binding but also the ability of the agonist to produce a response once bound. He coined the terms 'affinity' for the binding step and 'efficacy' for the production of response once bound. He

rightly saw that drug development could be placed on a rational basis only if these two quantities could be measured separately, and would doubtless have realized that the same was true for studies in which receptor structure is changed by mutations, rather than agonist structure being changed.

Stephenson's article was very influential among pharmacologists, although widely misunderstood. It is, perhaps, not surprising that it was misunderstood because the theoretical structure that he proposed was simply wrong [29,30]. As a consequence, the methods that he and others proposed for measuring affinity and efficacy gave the wrong answers. This problem was propagated into much work that followed: for example, the 'operational model' of James Black and Paul Leff [31] and related approaches of, for example, Terry Kenakin [32]. These proposals were very similar to that of Stephenson and did not postulate any sort of mechanism. They were empirical rather than physical approaches, and, like Stephenson's approach, could not separate 'affinity' as a physical quantity from subsequent events [30]. The ideas that Stephenson postulated remain relevant, and much of the work that used his ideas was undoubtedly valuable for drug development at an empirical level. But for the purposes of understanding how agonists work it was a dead end. The null ('constant response') methods that served Schild so well for antagonists could not, contrary to Stephenson's hope, be extended to agonists. This era is dealt with in more detail elsewhere [30,33]. It turned out that it was essential to consider reaction mechanisms explicitly to get anywhere, and that is where the subsequent progress lay. Biophysical, biochemical and, later, structural investigations of agonist action took over from Stephenson's abstract approach.

On the biophysical side, the major advances were made in the 1950s and 1960s by Wyman and by Katz.

Jeffries Wyman (1901–1995)

I think that, with hindsight, it could be argued that Wyman made a greater contribution to the understanding of conformational changes in receptors than any of the others mentioned here, although he never actually worked on receptors. He is the only American in my list of heroes, although the UCL connection seems inescapable even in his case. Wyman left Harvard Graduate School in 1924, and sailed on a slow steamer for England with John Edsall, 200 other passengers and 700 cattle ([34]; http://stills.nap.edu/html/biomems/jwyman.html). In Cambridge he met Gowland Hopkins, Gilbert Adair and J.B.S Haldane, among others, but soon left for UCL to get his PhD, under the supervision of Hill.

In 1951, he published a paper [35] that had enormous influence. It made the tentative suggestion that observations on the binding of oxygen to haemoglobin could be explained very economically if it were supposed that the two different conformations of haemoglobin (already known then) had different affinities for oxygen, so binding of oxygen would shift the conformational equilibrium towards the high-affinity form. This would explain the observed 'cooperativity' of oxygen binding without having to postulate an interaction between different binding sites. This idea was eventually applied to cooperative enzymes by Monod, Wyman and Changeux [36], and subsequently to nicotinic receptors [37]. Wyman's crucial role is evident from a private letter from Monod to Wyman concerning a draft of their 1965 paper [*Lettre de Jacques Monod à Jeffries Wyman le 29 mai 1964* (http://www.pasteur.fr/infosci/archives/mon/im_wym.html), which reads as follows:

> "Whatever they may be, my dear Jeff, I eagerly await your comments and suggestions. As you already know, and as I think you will see in this current version, the whole paper is inspired and permeated by the ideas which you have expounded and perfected over many years. After your revision, I think your influence will be still stronger and I may say that it will be for me a very great honour, as well as a great pleasure to be an author of this paper alongside you." Jacques Monod (translated by Margaret Colquhoun)

Although the Monod–Wyman–Changeux scheme has not proved to be sufficient to describe any ion channel (the only system that is simple enough to allow critical tests), Wyman's suggestion has recently been used to provide an elegant way to describe the apparent cooperativity in the glycine receptor without having to postulate an interaction between distant binding sites [38].

In 1959 Edsall and Wyman [39] treated the question of linked reactions and reciprocal effects in a very general way. If I had understood properly what they wrote then, it would not have taken until 1987 to see the flaw in Stephenson's formulation of the problem [29].

It was Wyman's work that led directly to the idea of 'two-state models'. These have been used widely to produce qualitative descriptions of phenomena like inverse agonism and spontaneous ('constitutive') activity of receptors ([30], and see later).

Another important development in the 1960s was the introduction of the direct measurements of the binding of radioligands to receptors. This was achieved by Bill Paton and Humphrey Rang [40] in a beautiful paper that is still well worth reading. The authors measured the binding of atropine to smooth muscle cells over a very wide range of concentrations, and used proper least-squares fitting on the results (at a time when using a computer was a major undertaking). They detected a component of binding that resulted from occupancy of muscarinic acetylcholine receptors, and were able to identify this component with some certainty because they knew, from the Schild method, what the affinity should be. No new mathematical principles were involved but the influence was enormous.

Bernard Katz (1911–2003)

Katz was appointed to the chair of Biophysics at UCL in 1952, shortly after Biophysics had, at last, become a separate department. Katz was Hill's successor at UCL and the high regard he had for Hill is very obvious [5]. Katz described his arrival in London thus:

> "I was born in March 1911 in the town of Leipzig in the middle of Germany. But I had a "re-birthday", 24

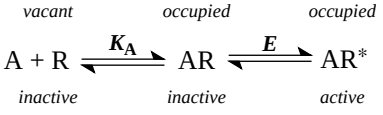
years later, when I arrived at the port of Harwich in England, one afternoon in February 1935. I had escaped from Hitler's Reich, and after a somewhat grueling interview with His Britannic Majesty's Officer of Immigration, I was allowed to enter the UK. The next day, I climbed a long staircase to the top floor of University College London and presented myself to Professor Hill. He received me like a new member of his scientific family. Having got away from dark and hostile surroundings, the contrast was a tremendous experience for me. I felt a little like David Copperfield when he arrived, bedraggled and penniless, at the home of his aunt and was put into a clean hot bath."

For some details of Katz's life and work, see [41,42] and http://www.physiol.ucl.ac.uk/Bernard_Katz/.

One of Katz's many enormous talents was his ability to reduce a problem to its essentials, without over-simplification or excessive speculation. At the end of his inaugural lecture in 1952, he said, in characteristically unpretentious style,

"My time is up and very glad I am, because I have been leading myself right up to a domain on which I should not dare to trespass, not even in an inaugural lecture. This domain contains the awkward problems of mind and matter about which so much has been talked and so little can be said…"

When faced with trying to explain the actions of partial agonists Katz took exactly the opposite approach to Stephenson. Rather than trying to provide a general, and somewhat abstract, theory, del Castillo and Katz proposed, in 1957, a specific and simple mechanism [43] that had, following the binding of agonist, a separate channel opening step:



This explicit separation of binding and gating was sufficient to remove all the ambiguities from earlier approaches, and Katz's mechanism forms the basis for most work that has been done since on the way that agonists cause channels to open. del Castillo and Katz's article published in 1957 can, perhaps, be regarded as the progenitor of most subsequent work on how agonists cause ion channels to open. There is no need to go into details here because this work has recently been reviewed extensively [30,44,45].

Katz can also be regarded as the 'father' of more-recent work on single ion channels. His work on noise analysis in the early 1970s was a major source of inspiration to Sakmann who was a postdoctoral fellow in Katz's department from 1970 to 1973 (http://www.physiol.ucl.ac.uk/Bernard_Katz/memories%20of%20bk-k%20sakmann_rev.htm). Realization of the goal of measuring single-ion-channel currents required, once again, the participation of a physicist, Erwin Neher [46]. Single-channel measurements offered a power to dissect mechanisms that was vastly higher than that of macroscopic measurements [47,48].

More-recent developments

It was soon realized that single-ion-channel observations offered a way of dissecting apart the two steps in receptor activation that del Castillo and Katz had proposed: the binding step and the gating step. But to achieve this required new theoretical work to be done. Up to now, the mathematics has been essentially trivial: all macroscopic phenomena, however complex the reaction mechanism, can be expressed in a single equation [49], the vector of state occupancies being $p(t)=p(0)\exp(Qt)$, where Q is the transition rate matrix (under conditions where concentrations, etc. are constant). But single molecules behave randomly, and, up to this time, the only people who had dealt with this sort of behaviour were particle physicists. The mathematics suddenly became by no means trivial, and again input was needed from mathematicians. This expertise was supplied initially by Alan Hawkes, who, in a series of papers, provided the groundwork for interpretation of single-channel measurements [50]. Some of the history of this period has been recorded [44]. As with every topic discussed in this paper, many others have contributed (e.g. Frank Ball [51]), but Hawkes pioneered the principles on which all later work depended [49,52,53]. He was also responsible for finding an exact solution to the problem that very brief events cannot be detected [54,55], which allowed the development of optimum fitting methods for single-channel data [56,57], and for work on non-stationary single channels [47]. As a result of his work it has become possible to measure as many as 18 rate constants from a single set of ion-channel recordings [38], a resolution undreamt of in studies on enzymes or on G-protein-coupled receptors. Efforts are now concentrated on dissecting the intramolecular movements that lead from binding to gating [38,58–60].

Attempts to make similarly detailed studies on G-protein-coupled receptors have, so far, proved impossible. Although reaction mechanisms have been proposed that are based on physical considerations [61–63], the information is simply not there to identify even equilibrium constants, never mind rate constants. Therefore, quantitative tests of the proposals are not possible. One of the biggest problems is the almost complete lack of knowledge about what happens to the receptor itself. All information comes from events further downstream in the transduction pathway but, without knowledge of the conformation change in the receptor itself, it is impossible to say, for example, why a partial agonist is 'partial'. Another major problem is that the equations all have steps that involve G-protein binding to the receptor but the concentration of the G protein is unknown; in fact insofar as it is membrane bound, it is not known how to express concentrations at all. An early, and intriguing, observation on G-protein-coupled receptors was that agonist binding curves seemed to show multiple binding components in the absence of GTP, but that addition of GTP converted agonist binding mostly to the low-affinity form [64]. This must be telling us something interesting but, a quarter

of a century after the original observations, it is still not certain why it happens.

Postscript

Pharmacology started as a branch of therapeutics, and that is still one important aspect of the subject. Alongside that, during the past 100 years, pharmacology has become a quantitative subject with a sound basis in the physical sciences. At the same time, enormous amounts have been learned about transduction mechanisms. The most common sort of receptor, the G-protein-coupled receptor family, has turned out to be more complex than Clark could have imagined. To this day, it is not possible to describe their response to agonists in the mathematical way that he might have hoped: to do so will need some radical developments in experimental methods. Clark said:

> "In the first place, there is no advantage in fitting curves by a formula unless this expresses some possible physico-chemical process, and it is undesirable to employ formulae that imply impossibilities. It is a question of finding a few systems so simple that it is possible to establish with reasonable probability the relation between quantity of drug and the action produced…" [12]

The only systems that have proved to be "so simple that it is possible to establish with reasonable probability the relation between quantity of drug and the action produced" are a few agonist-activated ion channels, and in that area great progress has been made. Indeed, in some ways that field, thanks to the ability to observe single molecules, has far surpassed enzymology and protein chemistry, in which it is still far from possible to measure 18 rate constants from a single set of experiments. The history outlined here makes it clear that the development of the subject has been dependent on input from physical scientists and mathematicians. In fact, progress might have been much quicker if Clark and Gaddum had interacted more with enzymologists and physical chemists (who understood competitive actions far earlier than pharmacologists). Likewise, if Stephenson had known about Katz, and if both had known about Wyman's work on haemoglobin, much misunderstanding could have been avoided.

The moral, perhaps, is that the best way to get on in science is to waste more time drinking coffee with colleagues from other subject areas. That, at least, has served me well.

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