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REVIEW ARTICLE

The Hill equation: a review of its capabilities in pharmacological modelling

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ABSTRACT

The Hill equation was first introduced by A.V. Hill to describe the equilibrium relationship between oxygen tension and the saturation of haemoglobin. In pharmacology, the Hill equation has been extensively used to analyse quantitative drug–receptor relationships. Many pharmacokinetic–pharmacodynamic models have used the Hill equation to describe nonlinear drug dose–response relationships. Although the Hill equation is widely used, its many properties are not all well known. This article aims at reviewing the various properties of the Hill equation. The descriptive aspects of the Hill equation, in particular mathematical and graphical properties, are examined, and related to Hill's original work. The mechanistic aspect of the Hill equation, involving a strong connection with the Guldberg and Waage law of mass action, is also described. Finally, a probabilistic view of the Hill equation is examined. Here, we provide some new calculation results, such as Fisher information and Shannon entropy, and we introduce multivariate probabilistic Hill equations. The main features and potential applications of this probabilistic approach are also discussed. Thus, within the same formalism, the Hill equation has many different properties which can be of great interest for those interested in mathematical modelling in pharmacology and biosciences.

INTRODUCTION

The basic expression of what is called the Hill equation (or model) is a three parameter equation of a nonlinear relationship between two variables, x (the independent variable) and y (the dependent variable):

$$y = \frac{y_{\max} x^{\alpha}}{c^{\alpha} + x^{\alpha}} \qquad (0)$$

The three parameters of the equation are $y_{\max}$, c and the coefficient α. This equation is numbered zero for ease of comprehension of the next part.

The first application of Eqn (0) in pharmacology was presented by A.V. Hill [1] in 1910. Since then, the Hill equation (or a similar one) has been used to fit experimental data from various physicochemical reactions: enzymatic reactions (the Michaelis–Menten equation [2]), or acetylcholine on muscular cells [3] for example.

In pharmacology, on the rational basis of the receptor occupancy theory [4], the Hill equation was proposed by Wagner [5] as a model for describing the drug concentration–effect relationship. Since then, in the last three decades, pharmacokinetic–pharmacodynamic (PK–PD) modelling has been developed to describe the complex relationships between drug doses, drug concentrations and drug effects over time. The Hill model has been extensively used when the relationship between drug

concentration and drug effect is nonlinear and saturable [6,7].

The main reason for the success of the Hill model is probably its flexibility and effectiveness in fitting experimental data. Because of this, many studies in pharmacology have used what can be called the descriptive properties of the Hill model.

Going back to its origins, the Hill model is very rich and exhibits many other interesting aspects and properties. Considering this, a systematic use of the Hill model as a curve fitting tool seems a bit limiting. This overview, which considers various properties of the Hill equation, will be divided into four parts:

1. Presentation and exploration of the descriptive and deterministic properties of the Hill model in biosciences. Hill's article is used as an example to present these aspects.

2. Examination of the mechanistic features of the Hill equation in a setting of physicochemical equilibrium.

3. Presentation of the probabilistic aspects of the Hill equation. In this section, we also develop some new mathematical results and suggest interesting potential applications of a probabilistic Hill equation in pharmacology.

4. Finally, we discuss the differences, the relationships and the boundaries between these various capabilities of the Hill equation.

As this overview focuses on the quantitative and modelling properties of the Hill equation, it contains many mathematical equations. For ease of reading, only the most relevant expressions are presented in the text. More details can be found in the Appendix.

DESCRIPTIVE PROPERTIES OF THE HILL EQUATION

History: the three Hill equations

In 1910, Archibald Vivian Hill (1886–1977) described quantitative equilibrium relationships between oxygen tension (pO_2) and the percent saturation of haemoglobin (Hb) with oxygen. His purpose was to find a useful equation to describe different experimental observations of Hb saturation by oxygen in solutions of various salts. In his original publication, he proposed three mathematical expressions to describe these relationships [1]. They can be plotted to obtain the so-called oxygen–Hb dissociation curve.

Among his three equations, what is now called the Hill equation was his B equation:

$$y_1 = 100\frac{Kx^{\alpha}}{1 + Kx^{\alpha}} \quad (1)$$

where y_1 represents the percentage of Hb saturation with oxygen, and x the partial pressure of O_2 (mmHg). K and α are the two parameters of this equation.

Hill was concerned that the molecules of Hb might aggregate differently because of the various salts present in solution. Because of this, as different aggregates of Hb might co-exist in the same solution, several simultaneous equilibrium reactions between oxygen and Hb might co-exist as well. If a solution would contain only Hb and Hb_n (an aggregate of n molecules of Hb), the equilibrium reaction would be:

$$Hb + O_2 \leftrightarrows HbO_2$$
$$Hb_n + nO_2 \leftrightarrows Hb_nO_{2n}$$

In the special case of a solution with only Hb and Hb_2, the equation of the dissociation curve of Hb proposed by Hill was his A equation:

$$y_2 = \lambda\frac{K_2x^2}{1 + K_2x^2} + (100 - \lambda)\frac{K_1x}{1 + K_1x} \quad (2)$$

where y_2 is the percent saturation of Hb, x is the partial pressure of O_2 (mmHg), λ is the percentage of Hb_2, and $(100 - \lambda)$ is the percentage of Hb. K_1 and K_2 were called by Hill the 'equilibrium constants' of the equilibrium reactions.

A third equation presented by Hill was a general one considering the co-existence of n types of aggregates and consequently n equilibria with oxygen:

$$y_3 = \sum_{r=1}^{n}\lambda_r\frac{K_rx^r}{1 + K_rx^r} \text{ with } \sum_{r=1}^{n}\lambda_r = 100, \quad \lambda_r \geq 0 \quad (3)$$

where y_3 is the percent saturation of Hb, x is the partial pressure of O_2 (mmHg) and λ_r is the percentage of each Hb_r aggregate.

Mathematical and graphical properties of the Hill equation

With a first set of observations, Hill calculated and plotted the percent saturation of Hb using Eqn (2) and he compared his results with those calculated using Eqn (1) and with experimental results found by Barcroft and Camis [1].

Both the results obtained with Eqns (1) and (2) were close to their observations, but if Hill plotted the whole curve of Eqn (2), he only presented the Hb saturation values predicted by Eqn (1).

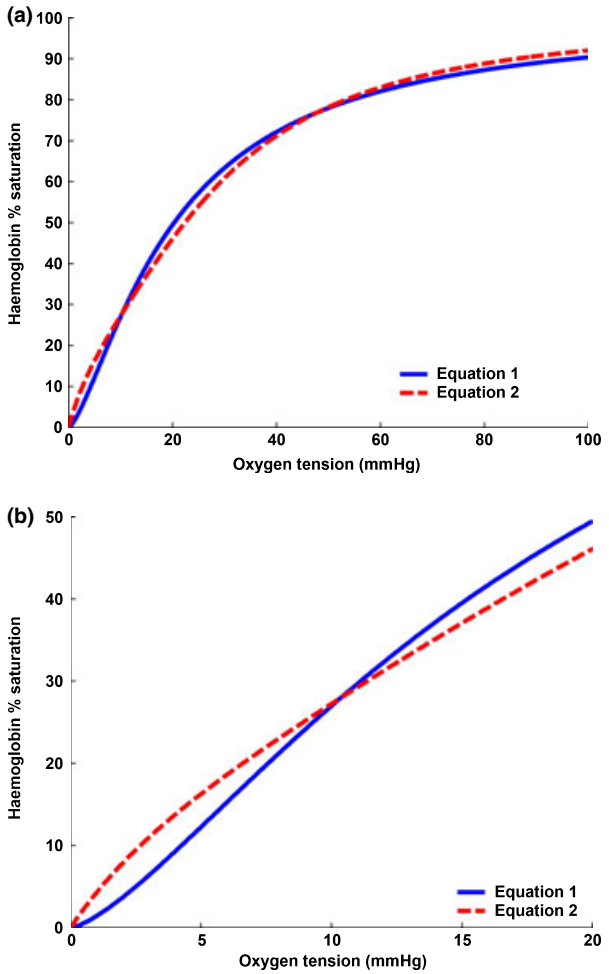

Figure 1 Dissociation curves of haemoglobin according to Hill Eqns (1) and (2). (a) Overall aspect; (b) lower parts of the curves. The parameter values used are those given by Hill: $\alpha = 1.405$ and $K = 0.01455$ for Eqn (1), $\lambda = 62$, $K_1 = 0.125$, $K_2 = 0.0011$ for Eqn (2). Note the different shapes of the curves in their lower parts.

Figure 1 shows the plots of Eqns (1) and (2). The parameter values used here are those given by Hill [1]. It should be noted that the graphical shape of the plots of Eqns (1) and (2) are in fact quite different in their lower ranges. Hill did not explore this difference because he did not use any value of oxygen tension less than 10 mmHg.

The plot of Eqn (1) (Hill's B equation) has a typical sigmoid shape. The existence of an inflexion point can be demonstrated by calculating the first and second derivatives of Eqn (1) (see Appendix). One can show that the abscissa of the inflexion point is

$$x = \left[\frac{\alpha - 1}{K(\alpha + 1)}\right]^{1/\alpha}$$

This inflexion point only exists if $\alpha > 1$. It leads to $x = 5.7138$ with $\alpha = 1.405$ and $K = 0.01455$, the parameter values given by Hill [1].

As the results calculated with both equations were close and were consistent with experimental results, Hill chose to use Eqn (1) (his B equation) to describe other sets of observations. The reason for this was the ease of calculation for curve-fitting purposes. Fitting data with Eqn (1) only requires the estimation of two parameters (α and K) while Eqn (2), generalized in Eqn (3), requires the estimation of $2n - 1$ parameters.

Equation (1), the original Hill model, represents here a curve-fitting descriptive model. It can be obtained from the general expression of the Hill equation, i.e. Eqn (0), by setting $c = K^{-1/\alpha}$ and $y_{max} = 100$. Parameter estimation can lead to any real values for K and α in Eqn (1). These values are generally informative. K [or c in Eqn (0)] may reflect the affinity and/or the sensitivity of the system, while α may indicate heterogeneity in the response. However, they give no information about the mechanisms involved. Equation (1) [as well as Eqn (2)] can also be called a deterministic or pseudo-deterministic model, because if one assumes that such a model correctly describes a system and if one is able to get measurements which permit estimation of the model parameter values, under defined conditions, it then would be possible to predict the state of the system under other conditions.

Past and current uses of the deterministic Hill model

Biology

As a descriptive-deterministic model, the Hill equation has been used extensively in biosciences. In enzymology, the Michaelis–Menten equation [2], which describes the relationship between the velocity of a reaction v and the concentration of substrate $[S]$ for a single substrate–enzyme interaction, has the following expression:

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (4)$$

where v is the initial velocity of the reaction (mol/L/s), V_{max} is the maximum reaction rate (mol/L/s), $[S]$ is the substrate concentration (mol/L), and K_m is the concentration of substrate at which the rate of the enzymatic reaction is half V_{max} (mol/L). Equation (4) may be obtained from Eqn (0) by replacing x, y, c, y_{max} and α by $[S]$, v, K_m, V_{max} and 1 respectively. Thus, mathematically, the Michaelis–Menten equation is a special example of the Hill equation. Note that the nature

of the effect described here is different from the one considered by Hill, as v is a dynamic effect, while Hb saturation is a static or steady-state effect. However, an equilibrium physicochemical reaction is involved in both settings.

In physiology, the Hill equation has been used to describe nonlinear and saturable mechanisms, for example the renal uptake of aminoglycosides by proximal tubular cells [8,9], the tubuloglomerular feedback of the glomerular filtration in the kidney [9,10], and the effect of ligand binding on the conductance of voltage-dependent ion channels [11,12].

Pharmacokinetic–pharmacodynamic modelling

Focusing on pharmacodynamics, the Hill equation has been widely used to describe the relationship between drug effect (E) and drug concentration (or drug dose) (C), in the so-called 'sigmoid E_{max} model' [13,14]. Mathematically, this model is simply a rewriting of the general Hill equation. It is obtained from Eqn (0) by substituting the two variables x and y by C and E, respectively, and the parameters c and y_{max} by EC_{50} and E_{max}, respectively, as follows:

$$E = \frac{E_{max} C^{\alpha}}{EC_{50}^{\alpha} + C^{\alpha}} \quad (5)$$

E is the predicted effect of the drug, E_{max} is the maximum effect, C is the drug concentration at time t, EC_{50} is the drug concentration for which 50% of maximum effect is obtained and α is the Hill coefficient of sigmoidicity. In pharmacology, the unit of drug effect E depends on the response considered; the unit of drug concentration C and EC_{50} is usually expressed in mol/L or g/L, and α has no unit.

Note that the effect can be either static or dynamic. When drug concentration changes with time, the related effect may change too, and one of the goals of PK–PD models is to describe the relationship between concentration and effect over time.

In some situations, it is necessary to include a baseline response E_0 (effect at a drug concentration of 0) in the analytical expression. This transforms Eqn (5) into a general four-parameter Hill equation:

$$E = E_0 + \frac{E_{max} C^{\alpha}}{EC_{50}^{\alpha} + C^{\alpha}} \quad (6)$$

The effect to be modelled can be either positive or negative (i.e. the observed response may increase or decrease with rising concentrations). To describe a negative effect, a monotonically decreasing Hill model may be obtained by considering a negative Hill coefficient of sigmoidicity α, or a minus sign before the second right term in Eqn (6).

The effect may be expressed as a fraction or a percentage of maximum effect E_{max}, and so Eqn (6) becomes:

$$E/E_{max} = E'_0 + \frac{C^{\alpha}}{EC_{50}^{\alpha} + C^{\alpha}} \quad (7)$$

where $E'_0 = E_0/E_{max}$.

As a consequence, this transformation leads to a normalization of the y-axis on the plots $E = f(C)$. Of course, it is impossible to do this when the maximum effect cannot be estimated. *Figure 2* shows plots of different concentration–effect Hill equations according to Eqns (5) and (6), with different values of the parameters α, c and E_0.

Another common graphical representation is the semi-logarithmic one, on the x-axis, as shown in *Figure 3*. The graphs obtained are similar to the classical dose–response curves in pharmacology.

Moreover, using the fraction expression of the effect as described above, and a logarithmic transformation, a linearization of the Hill equation is possible.

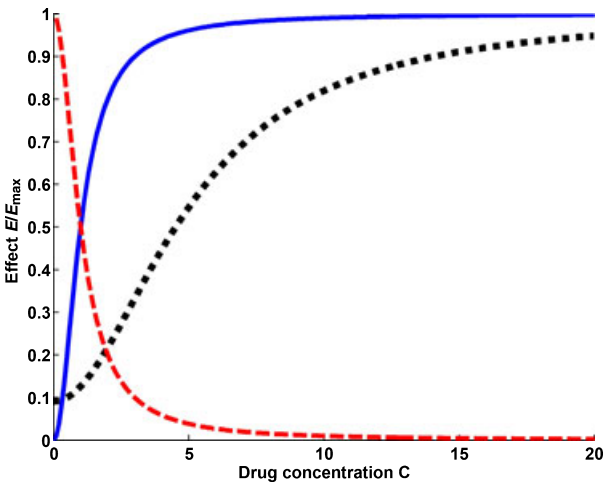

Figure 2 Different types of concentration-effect Hill equations. Solid line: equation for no baseline effect [Eqn (5) was used with parameter values $\alpha = 2$ and $EC_{50} = 1$]; dashed line: equation for an inhibitory effect [Eqn (5) was used, with parameter values $\alpha = -2$ and $EC_{50} = 1$]; dotted line: equation for a baseline effect [Eqn (6) was used, with parameter values $\alpha = 2$, $c = 5$, $E_0 = 0.1$]. The effect is represented as a fraction of maximum effect (i.e. E/E_{max}). Units of drug concentration are only relative.

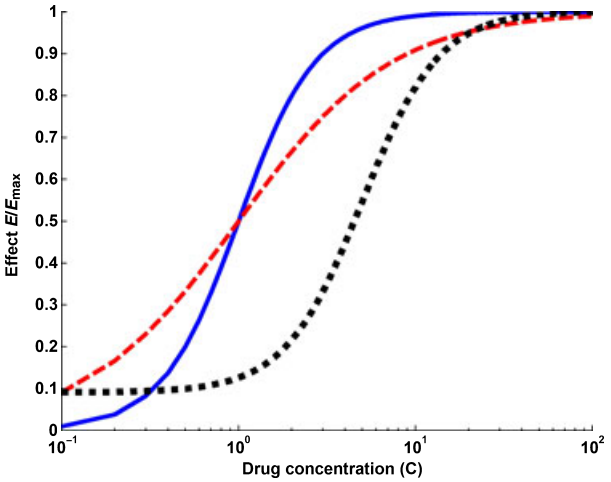

Figure 3 Semi-logarithmic plots of concentration-effect Hill equations.
Solid line: Eqn (5) was used with the parameter values $\alpha = 2$ and $EC_{50} = 1$; dashed line: Eqn (6) was used with parameter values $\alpha = 1$, $EC_{50} = 1$, $E_0 = 0.1$; dotted line: Eqn (6) was used, with parameter values $\alpha = 2$, $EC_{50} = 5$, $E_0 = 0.1$. The effect is represented as a fraction of maximum effect (i.e. E/E_{max}). Drug concentration is in $\log_{10}$ scale. Units of drug concentration are only relative.

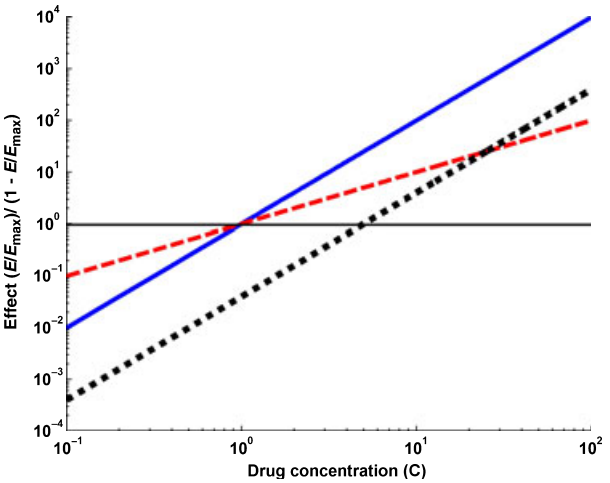

Figure 4 Median effect plots of concentration-effect Hill equations. The logarithmic transformation defined in Eqn (9) (see text) was applied to Eqns (5) and (6). Solid line: transformed Eqn (5) was used with parameter values $\alpha = 2$ and $EC_{50} = 1$; dashed line: transformed Eqn (5) was used with parameter values $\alpha = 1$ and $EC_{50} = 1$; dotted line: transformed Eqn (6) was used with parameter values $\alpha = 2$, $EC_{50} = 5$, $E_0 = 0.1$. Both drug concentration and effect are in $\log_{10}$ scale. This type of plot may give the value of α (slope) and EC_{50} which is the value of concentration for which $y = 0$.

Actually, if E_0 is equal to zero, we have:

$$\frac{E/E_{\max}}{1 - E/E_{\max}} = \left[\frac{C}{EC_{50}}\right]^{\alpha} \tag{8}$$

Finally, a logarithmic transformation yields:

$$\log\left(\frac{E/E_{\max}}{1 - E/E_{\max}}\right) = \alpha \log(C) - \alpha \log(EC_{50}) \tag{9}$$

Note that one can use either natural or decimal logarithm, but the use of decimal logarithm is widespread in dose (or concentration)–response studies.

Equation (9) is known as the median-effect equation [15]. It permits the estimation of α (slope) and EC_{50}, when such a model fits properly a set of data, by linear regression for example. As an illustration, *Figure 4* shows plots of the median-effect equation [Eqn (9)] for various parameter values.

Even though useful, a logarithmic model such as Eqn (9) should be used with caution for several reasons cited by Holford and Sheiner [13]. First, it has been shown that linear regression is not the most precise method in estimating parameters of a Hill equation (weighted nonlinear regression is better for this purpose, see [16]). Second, the two parameters of Eqn (9), graphically the slope and the intercept, do not have a direct physical meaning, beyond their formal one. Third, Eqn (9) has no solution when the concentration C is 0, and it has no maximum finite value, contrary to Eqn (5). As minimum and maximum effects appear explicitly with Eqn (5) or Eqn (6), these expressions are usually preferred to the logarithmic form [Eqn (9)] in pharmacology.

Applied to anti-infectious drugs in microbiology, the Hill equation can be used to build pharmacodynamic models of the activity of antibiotics against microorganisms. An application of the Hill equation developed by Zhi et al. is shown below [17–19]:

$$\frac{dN}{dt} = N\left(G - \frac{E_{\max} C_t^{\alpha}}{C_{50}^{\alpha} + C_t^{\alpha}}\right) \tag{10}$$

where N is the bacterial population size (CFU/mL), G is the rate constant of exponential growth without exposure to the antibiotic (h^{-1}), E_{max} (h^{-1}) is the maximum bactericidal effect, α is the Hill coefficient of sigmoidicity, C_t is the antibiotic serum concentration (mg/L) at time t, and C_{50} is the antibiotic concentration producing 50% of E_{max} (mg/L).

Note that in the Zhi model, the effect is dynamic and the Hill equation is no longer a term of state equilibrium

equation but has become a term of a differential equation. A recent refinement of this model has been proposed by Mouton and Vinks [20].

Modelling the effect of combined therapy

Combined therapy is the rule for the treatment of many diseases, in particular infectious diseases like tuberculosis, AIDS, infectious endocarditis, severe lung infections and many others. In this case, concentration–effect modelling of the response must consider the effect E as a function of multiple variables (at least two).

The response surface model proposed by Greco et al. is probably the most well-known example of such an approach [21,22]. For a two-drug combined therapy, the modelled effect E is defined by an implicit equation:

$$1 = \frac{C_1}{\mathrm{EC}_{50,1}\left[\frac{E-B}{E_{\mathrm{con}}-B}\right]^{1/\alpha_1}} + \frac{C_2}{\mathrm{EC}_{50,2}\left[\frac{E-B}{E_{\mathrm{con}}-B}\right]^{1/\alpha_2}} + \frac{\gamma C_1 C_2}{\mathrm{EC}_{50,1}\mathrm{EC}_{50,2}\left[\frac{E-B}{E_{\mathrm{con}}-B}\right]^{(1/2\alpha_1)+(1/2\alpha_2)}} \quad (11)$$

where E is the combined effect, C_1 and C_2 are the concentrations of drugs 1 and 2, respectively, E_{con} is the baseline effect (no drug), B is the extrapolated background effect (maximum effect) at infinite drug concentration, $\mathrm{EC}_{50,1}$ and $\mathrm{EC}_{50,2}$ are the median effective concentrations for drugs 1 and 2, α_1 and α_2 are the Hill coefficients of sigmoidicity for drugs 1 and 2, and γ is the synergism–antagonism parameter.

In this expression, the basic model is again a Hill equation for a single drug, with four parameters, as defined in Eqn (6). Other response surface approaches based on the fundamental Hill model have been described [23,24].

Finally, even though much more complex PK–PD models have been built, the Hill model still remains the fundamental analytical expression for many of these models [6,7]. The prediction of a maximum effect, which is a key feature of biological phenomena, is probably one of the main reasons for the widespread use of the Hill model. The second reason appears to be the great flexibility of the model in fitting data, due to the shape parameter α, the Hill coefficient of sigmoidicity.

Pharmacological interpretation of the Hill equation

When the Hill model is used in PK–PD modelling, its parameters rarely have a mechanistic meaning, i.e. their values give no information about the physicochemical reaction involved. However, the mathematical equation itself may express the way the drug action is described. For example, in the antibacterial model described above [Eqn (10)], it is assumed that the antibiotic can only kill bacteria but cannot modify their growth. The confusing expression 'mechanism-based model' is often used to qualify such models. However, the confirmation of the so-called mechanism is hardly possible. Even if a good fit is obtained, different models can fit the same data well [25]. However, the estimated parameter values of the Hill equation may help characterize a specific effect of a drug. This allows comparing and classifying drug effects. For example, the Zhi model [Eqn (10)] has been applied to various antibiotic–bacteria couples [26]. Most of the time, the so-called time-dependent antibiotics such as beta-lactams have shown a high Hill coefficient value (α parameter) and a low maximum kill rate ($E_{\max}$ parameter) while the so-called concentration-dependent antibiotics such as aminoglycosides have been characterized by a low Hill coefficient and a high maximum kill rate [27–29]. In this way, both the effect of the so-called time-dependent and concentration-dependent antibiotics can be quantitatively described with the same structural Hill model. Only parameter values may be different. As a consequence, the most effective dosage regimen for an antibiotic may vary according to the parameter values of the Hill equation describing its effect [30].

MECHANISTIC PROPERTIES OF THE HILL EQUATION

Physicochemical equilibrium

Hill's experiments in 1910 dealt with the general setting of physicochemical equilibria. There is a strong connection between the Hill equation and the laws of equilibrium reactions. This relation was not mentioned in Hill's work [1] but was clearly stated a few years later by Clark [31] and McLean [32], for example.

As Hill did, let us consider the notion of a single equilibrium reaction, in a single liquid phase, between two molecules, L and M, and their combination LM

$$L + M \leftrightarrows LM$$

Derived from the law of mass action of Waage and Guldberg [33], the dissociation equilibrium constant K_{D} is defined as below:

$$K_D = \frac{[L][M]}{[LM]} \quad (12)$$

where $[L]$, $[M]$ and $[LM]$ are the molar concentrations of the three molecules.

In equilibrium conditions, we may define T as the entire concentration of molecule L, and y as the ratio of molecules L which have reacted. The expressions for T and y are given in Eqns (13) and (14):

$$T = [L] + [LM] \quad (13)$$

$$y = \frac{[LM]}{[LM] + [L]} \quad (14)$$

Substituting $[LM]$ by his expression from Eqn (12), we find:

$$y = \frac{[M]}{K_D + [M]} \quad (15)$$

Equation (15) is again a Hill equation derived from the general form given by Eqn (0) with two parameters $c = {K_D}^{1/\alpha}$ and $\alpha = 1$. In this setting, parameter c of the Hill equation is directly related to the K_D constant of the law of mass action. Moreover, one can note here that parameter K in the equation proposed by Hill [Eqn (1)] is the reciprocal of K_D.

As a consequence, in the setting of a single physicochemical equilibrium between three types of molecules, a Hill equation with two parameters describes the relationship between the proportion of molecules L which have reacted (y, ranging from 0 to 1) and the concentration of molecule M (x). The two parameters [c and α, see Eqn (0)] have a physical meaning beyond their formal one: α represents the number of binding patterns between M and L (see further below), and c is related to K_D which represents the concentration of M for which y is equal to 0.5 (i.e. $y_{max}/2$) and so it is an expression of affinity.

Considering this connection between the Hill equation and the law of mass action, it is not surprising to find a Hill equation (or a similar expression) describing various physicochemical equilibrium reactions: oxygen with Hb [1], adsorption of molecules on surfaces (the Langmuir isotherm [34]), enzymatic reactions [2], acetylcholine binding on muscular cells [3] and others [16]. These examples are some arguments for a relative universality of equilibrium principles. Various equations were described for various experimental settings. However, all of them feature an equilibrium system, and all these equations are related to the law of mass action [32,35].

Multiple binding patterns

Thereafter, the wide use of the Hill equation outside this context has shown some discrepancies in its mechanistic properties. Seeking to generalize the Hill equation to multiple binding patterns, one may consider the following equilibrium:

$$L_n + nM \leftrightarrows L_nM$$

For instance, if L is a receptor and Fn molecules of M are able to bind to the same receptor, then the related dissociation constant K_n is:

$$K_n = \frac{[L_n][M]^n}{[L_nM]} \quad (16)$$

and the Hill equation relative to the ratio $y_n = \frac{[L_nM]}{[L_nM]+[L_n]}$ is given by:

$$y_n = \frac{[M]^n}{K_n + [M]^n} \quad (17)$$

In this expression, the α parameter from Eqn (0) is represented by n, as n here can only take integer values. The assumption that α (or n) would represent the number of binding sites has been very tempting, as if one could estimate its value (by a curve-fitting approach for example), it would be possible to predict the number of binding sites on a specific receptor.

Clark [3] was the first to use the Hill equation in pharmacology. His experiments involved the contraction of smooth muscle cells by acetylcholine. In 15 experiments, the estimations of parameter K showed a great variability (from 1.6×10^6 to 33×10^6) when the α parameter showed only a slight variability (from 0.8 to 1.1). Clark concluded that the possible value for the α parameter was close to 1. The simplest explanation given by Clark was the existence of a reversible monomolecular relation between acetylcholine and the cellular receptors. However, Clark did not exclude the fact that the value of the α parameter might be different from 1. In that case, many other types of reactions could be described. In 1968, Wagner [5] observed that, in certain experimental conditions, the α value can be close to 2, which suggested a possible trimolecular reaction.

However, it has been shown thereafter that the Hill coefficient is not a correct estimate for the number of binding sites except in a very specific pattern of multiple interactions called positive cooperativity [36]. When

several molecules of ligand bind to a receptor, the value of the Hill coefficient is usually less than the number of binding sites. Therefore, its meaning is far less informative, but may reflect the extent of cooperativity between the binding sites [36].

As a consequence, the proper expression of a general Hill equation for physicochemical equilibrium is Eqn (18) rather than Eqn (17):

$$y_\alpha = \frac{[M]^\alpha}{K_\alpha + [M]^\alpha}, \quad \alpha > 0 \tag{18}$$

Finally, it should be noted here that we have gone back to Hill's central issue again. This can be considered as a slight formal but nevertheless an important theoretical shift from a mechanistic explanatory model (Eqn (17), with an integer valued coefficient n) to a descriptive curve-fitting model (Eqn (18), with a real valued coefficient α).

PROBABILISTIC ASPECTS OF THE HILL EQUATION

Definition

A probabilistic use of the Hill equation is possible as the term $x^\alpha/(c^\alpha + x^\alpha)$ can be the expression of a cumulative distribution function $F(x)$ for a random variable X [37], i.e.

$$F(x) = P\{X \le x\} = \frac{x^\alpha}{c^\alpha + x^\alpha}, \quad x \in R^+, \ c > 0 \text{ and } \alpha > 0 \tag{19}$$

Another common presentation is:

$$F(x) = \frac{(x/c)^\alpha}{1 + (x/c)^\alpha} = 1 - \frac{1}{1 + (x/c)^\alpha} \tag{20}$$

The related probability density function $f(x)$ is given by:

$$f(x) = F'(x) = \frac{\alpha\, c^\alpha x^{\alpha-1}}{(c^\alpha + x^\alpha)^2} = \frac{(\alpha/c)(x/c)^{\alpha-1}}{[1 + (x/c)^\alpha]^2} \tag{21}$$

The Hill equations as defined above constitute a family of bi-parametric probability functions defined in the positive real numbers. The notation $F(\alpha, c)$ will be used further. Illustrative plots of $F(x)$ and $f(x)$ are presented in *Figures* 5 and 6 and show the influence of the shape parameter α and of the size parameter c on the aspect of each curve.

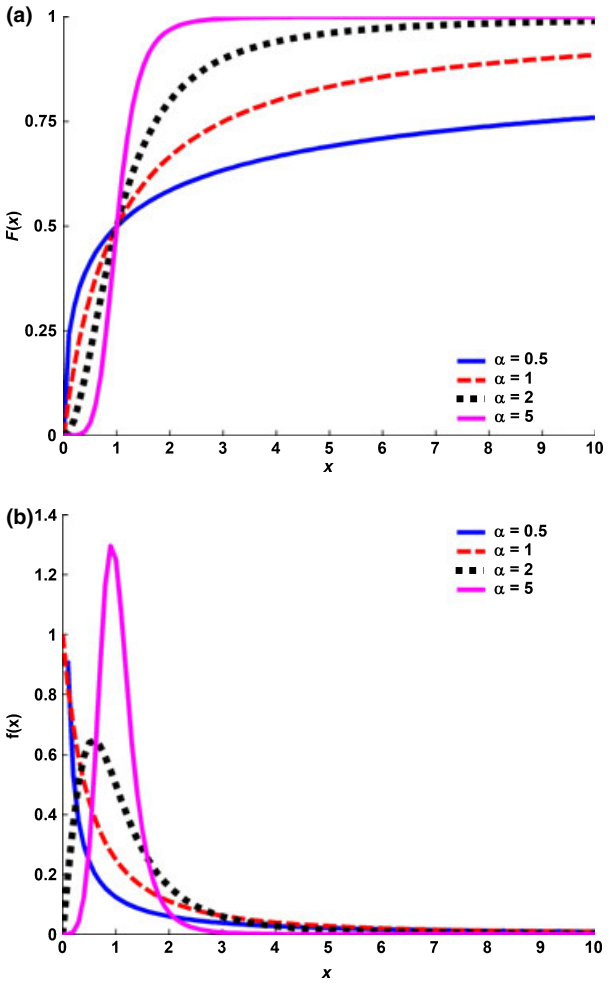

Figure 5 Influence of the shape parameter α on the Hill cumulative distribution function [$F(x)$ (a)], and on the Hill density function [$f(x)$ (b)].
$F(x)$ and $f(x)$ are defined by Eqns (19) and (21) respectively (see text). For parameter c, the value was set at 1.0. Note that the sigmoid shape of the Hill distribution function $F(x)$ appears only when $\alpha > 1$.

Equation (19) was studied previously by Burr, for example, in its overall scope of probabilistic cumulative distribution functions [37]. Basic results such as calculation of moments and quantiles are detailed in the Appendix.

Logit transformation

Considering the event $\{X \le x\}$ for a random variable X, if we set $p = P\{X \le x\}$, the probability of this event, the logit function is defined by the following expression:

$$\text{logit}(p) = \ln\left(\frac{p}{1-p}\right) \tag{22}$$

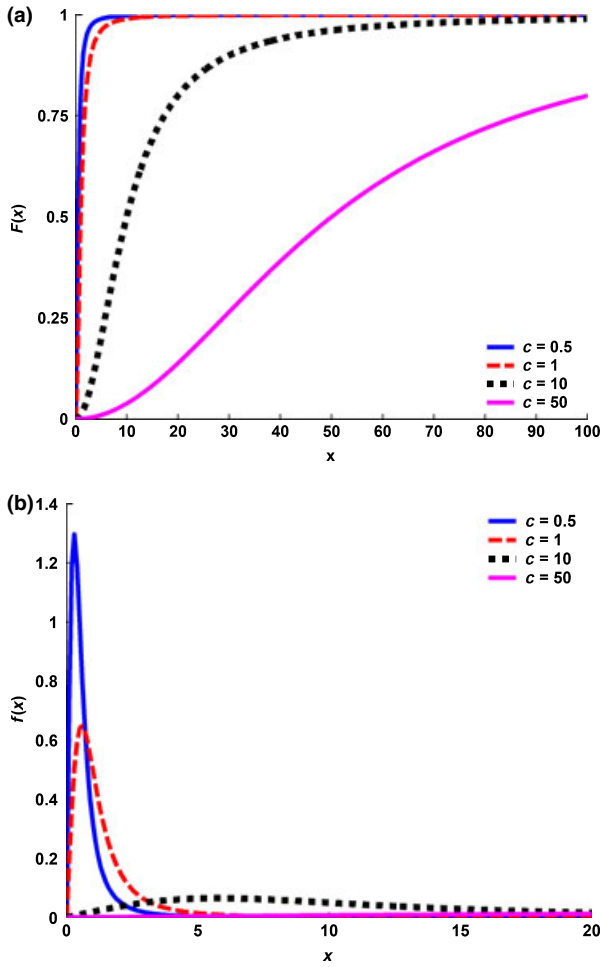

Figure 6 Influence of the size parameter c on the Hill cumulative distribution function [$F(x)$ (a)], and on the Hill density function [$f(x)$ (b)].
$F(x)$ and $f(x)$ are defined by Eqns (19) and (21) respectively (see text). For α, the parameter value was fixed at 2.0.

Applied to the Hill distribution [Eqn (19)], it leads to:

$$\text{logit}(p) = \ln\left(\frac{p}{1-p}\right) = \alpha(\ln x + \ln c) \qquad (23)$$

Therefore, the logit transformation leads to a linear form of the probabilistic Hill equation. Mathematically, the logit expression is similar to the median effect equation of Chou et al. cited previously [Eqn (9)], in their different setting of dose–effect relationship [15].

Fitting a probabilistic Hill equation to data, and parameter estimation

Fitting a probabilistic Hill equation to data and estimating its parameter values requires no specific approach. In order to demonstrate that observed values x_i of a random variable X are described by a probabilistic Hill equation $F(\alpha, c)$, one needs only to follow a classical assumption – deduction process using the appropriate statistical tests, such as Pearson's chi-squared test or maximum likelihood methods.

Note that a preliminary visual examination may be performed, using the relationships described between parameters and quantiles. A quantile x_q of a distribution function is the solution of the equation $F(x_q) = q$ (q ranging from 0 to 1). Using the Hill distribution function [Eqn (19)], it can be shown that (see Appendix)

$$\alpha = 2\frac{\ln[q/(1-q)]}{\ln(x_q/x_{1-q})}$$

and

$$c = (x_q x_{1-q})^{1/2}.$$

Thus, one can check on a graph that c and α remain constant for any q between 0 and 1. The estimation of the parameters α and c may be made using a classical approach like least square regression or maximum likelihood estimation, for example.

Current and potential applications

The Hill equation has been used to build probabilistic models in various fields. In physiology, gating models of ion channels [38,39] and models of decompression sickness in humans and animals [40] have used the probabilistic Hill equation.

Contrary to the descriptive approach, probabilistic uses of the Hill equation have been relatively rare in pharmacology, even if the notions of 'cumulative distribution function' and 'probability density function' have been known for decades [13,41–43]. The model of aminoglycoside nephrotoxicity published by Rougier et al. [9,44] is an example of such an application. The model relates the probability of occurrence of nephrotoxicity to the cumulative area under the time concentration curve of amikacin serum concentrations (AUC). The variable x of the Hill model [Eqn (19)] is noted AUC in this application and the cumulative distribution function is equal to:

$$F(\text{AUC}) = \frac{\text{AUC}^{\alpha}}{c^{\alpha} + \text{AUC}^{\alpha}} \qquad (24)$$

AUC and c are expressed in mg/h/L.

Modelling drug-induced toxicity is an interesting potential application of the probabilistic Hill equation. In this way, the Hill model may permit estimation of

the probability of an adverse event for a given dosage regimen. Therefore, it can be a powerful tool in clinical decision making, to evaluate and to choose the most appropriate dosage regimen for patients, taking into account both the expected efficacy and toxicity, as suggested by Troche et al. [45]. In the simple case where one Hill equation would describe the dose – or concentration – effect relationship, and a second Hill equation would describe the dose – or concentration – toxicity relationship, the therapeutic range would lie between the two curves. This is basically the principle of the well-known therapeutic index in pharmacology [42].

This probabilistic modelling approach should be encouraged, and the Hill model might be, in some situations, a reasonable alternative to probit analysis [46] and also to logistic regression coming from the general linear model (GLM) framework [47].

However, one should avoid any confusion between those three probabilistic methods. They use different transformations and different probability functions. In the applications presented above, the Hill equation is used as a distribution function itself. The probit method uses the normal distribution function and has been mainly used to analyse quantal (or all-or-none) responses in biology [41,48]. The mathematical bases of probit analysis are briefly presented in the Appendix.

The logistic regression uses the logistic distribution function and is very useful in epidemiology for example. However, the logistic regression and the probabilistic Hill equation should not be considered as interchangeable approaches. The logit transformation might be the main application responsible for such confusion. That transformation allows the linearization of a Hill distribution function [see Eqn (23)]. The logit transformation can be applied in the same way to the logistic distribution function (see Appendix). However, the resulting mathematical expression is different from the one obtained with the Hill equation [Eqn (23)]: the logistic distribution function relates to x whereas the Hill distribution function relates to the natural logarithm of x. Moreover, the goals are usually quite different. The GLM aims mainly at testing the statistical significance of each factor, while the Hill model provides an explicit function to connect the probability of various events to the variation of one variable (and eventually more). In this way, the Hill model can be used in its basic form [Eqn (19)], and the logit transformation is not necessary.

New probabilistic insights

Here are presented some new results concerning the probabilistic properties of the Hill equation.

Calculation of Shannon entropy and Fisher information matrix

Shannon entropy and Fisher information are two major applications of information theory. They describe the behaviour of dynamic systems. There is a growing interest in these two information measures in theoretical sciences. Fisher information may be used to analyse the sensitivity of a model to its parameters [49]. Another potential application of Fisher information is optimal design in dose – or concentration – response studies [50,51].

The specific calculations of Shannon entropy and Fisher information applied to a Hill probability function are detailed in the Appendix.

Hazard rate function

When X is set as a time variable, the equation can be used to describe the time of appearance of events such as damage, breakdown, abnormality, failure or death. The process to be described is usually time dependent over a defined duration.

During the time interval $[x, x + \xi[$, the probability that the event occurs is actually a conditional probability, as one knows that the event has not occurred yet. When ξ gets close to zero, the expression of this probability is given by the function $L(x)$:

$$L(x) = \frac{f(x)}{1 - F(x)} \tag{25}$$

which may be called the hazard rate function.

The knowledge of the formal expression of the distribution function F for Hill distributions allows one to calculate this function.

Note that, for a Hill function, we have:

$$f(x) = (\alpha/x)F(x)[1 - F(x)] \tag{26}$$

One can then obtain a simple expression of the hazard rate function:

$$L(x) = \frac{\alpha F(x)}{x} = \frac{\alpha x^{\alpha-1}}{c^{\alpha} + x^{\alpha}} \tag{27}$$

The shape of the hazard rate function depends greatly on the values of the α coefficient, as shown in *Figure 7*. As L grows, it may reflect an ageing or a weakening of the process. When it declines, it may reflect a process which improves with time. When

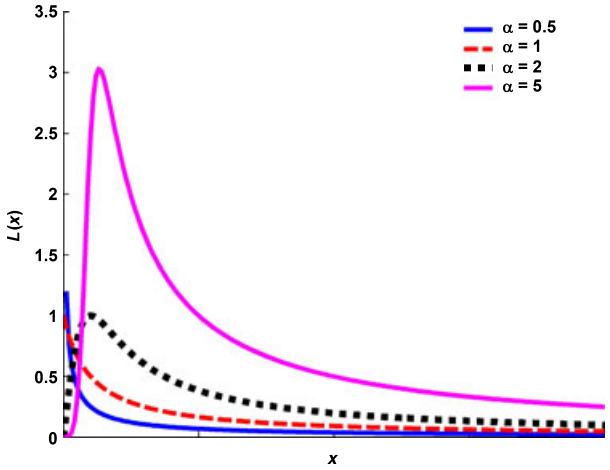

Figure 7 Plots of the hazard rate function $L(x)$ for various values of the α parameter.
The hazard rate function is defined by Eqn (27) (see text). The parameter value of c was fixed at 1.0. When $\alpha > 1$, the hazard rate function first grows to a maximum and then declines. It can be shown that $L(x)$ is maximum for $x = c\,(\alpha - 1)^{1/\alpha}$. When $\alpha = 1$, $L(x)$ declines monotonically, and its maximum is equal to $1/c$ when $x = 0$. When $0 < \alpha < 1$, the hazard rate function declines monotonically when x rises. When x gets close to zero, $L(x)$ gets close to infinity.

$\alpha > 1$, $L(x)$ first grows and then declines after a threshold value. The random process described is characterized by an increasing risk of the event at the beginning, the risk reaches a maximum and then declines after the threshold.

Multivariate probabilistic Hill equations

In the probabilistic use presented here, the Hill equation expresses the probability of the event $\{X \leq x\}$ for a single random variable X. Then, in the case of several random variables X_i ($i = 1,...,n$) and several events $\{X_i \leq x_i\}$, a joint Hill distribution equation can also be considered. This joint Hill distribution equation $F_n(x_1,...,x_n)$ expresses the probability of the intersection event, i.e.

$$F_n(x_1, \ldots, x_n) = P\left(\cap_{i=1,\ldots,n}\{X_i \leqslant x_i\}\right) \tag{28}$$

The expression of the related joint probability density function is [52]

$$f_n(x_1, \ldots, x_n) = \Gamma(n+1)\,\frac{\alpha_1/c_1(x_1/c_1)^{\alpha_1-1}\alpha_2/c_2(x_2/c_2)^{\alpha_2-1}\cdots\alpha_n/c_n(x_n/c_n)^{\alpha_n-1}}{[1+(x_1/c_1)^{\alpha_1}+(x_2/c_2)^{\alpha_2}+\cdots+(x_n/c_n)^{\alpha_n}]^{n+1}} \tag{29}$$

where $\Gamma(x)$ is the Euler function (gamma function).

Moreover, in this expression, each random variable X_i is characterized by a univariate Hill equation $F_i(\alpha_i, c_i)$. These univariate Hill distributions are basically the marginal distributions of the multivariate joint Hill equation. The covariance between any margin variable X_i and X_j is given by:

$$\mathrm{cov}(X_i, X_j) = c_i c_j \Gamma(1+1/\alpha_i)\Gamma(1+1/\alpha_j)\,[\Gamma(1-1/\alpha_i-1/\alpha_j) - \Gamma(1-1/\alpha_i)\Gamma(1-1/\alpha_j)] \tag{30}$$

Note that such covariance is defined only if the condition $1-1/\alpha_i - 1/\alpha_j > 0$ is respected.

In order to get the expression of the joint cumulative distribution function, one may use the expression of $F(x)$ given in Eqn (20), i.e.

$$F(x_i) = 1 - \frac{1}{1+(x_i/c_i)^{\alpha_i}}.$$

Then, for $n = 2$ for example, the expression of the joint cumulative distribution function is:

$$F_2(x_1, x_2) = 1 - \frac{1}{1+(x_1/c_1)^{\alpha_1}} - \frac{1}{1+(x_2/c_2)^{\alpha_2}} + \frac{1}{1+(x_1/c_1)^{\alpha_1}+(x_2/c_2)^{\alpha_2}} \tag{31}$$

And for $n = 3$, it is:

$$\begin{aligned} F_3(x_1, x_2, x_3) &= 1 - \frac{1}{1+(x_1/c_1)^{\alpha_1}} - \frac{1}{1+(x_2/c_2)^{\alpha_2}} - \frac{1}{1+(x_3/c_3)^{\alpha_3}} \\ &+ \frac{1}{1+(x_1/c_1)^{\alpha_1}+(x_2/c_2)^{\alpha_2}} \\ &+ \frac{1}{1+(x_1/c_1)^{\alpha_1}+(x_3/c_3)^{\alpha_3}} \\ &+ \frac{1}{1+(x_2/c_2)^{\alpha_2}+(x_3/c_3)^{\alpha_3}} \\ &- \frac{1}{1+(x_1/c_1)^{\alpha_1}+(x_2/c_2)^{\alpha_2}+(x_3/c_3)^{\alpha_3}} \end{aligned} \tag{32}$$

As an illustration, *Figure 8* shows the plot of a bivariate Hill distribution function as described in Eqn (31), with its two marginal distributions.

Finally, what we obtain here is an explicit analytical equation to describe the behaviour of a variable $y = F_n(x_1,...,x_n)$ as a function of several random variables X_i.

This equation might be used to build response surface models as the equation proposed by Greco et al. [21], i.e.

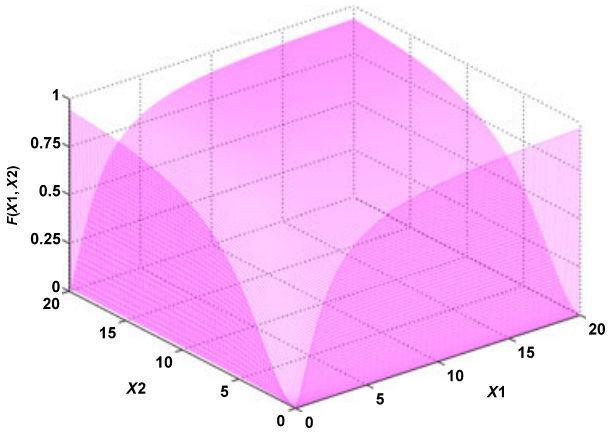

Figure 8 Bivariate Hill equation.
Equation (31) was used with the following parameter values: $\alpha_1 = 1.5$, $\alpha_2 = 2$, $c_1 = 2$, $c_2 = 5$. The margin Hill distributions of X_1 and X_2, i.e. $F(\alpha_1, c_1)$ and $F(\alpha_2, c_2)$, respectively, as defined in Eqn (19), are also plotted, using the same parameter values.

Eqn (11). This suggests potential applications for those equations in modelling the effect of multiple pharmacological agents used in combination.

DISCUSSION: UNITY OF THE HILL MODEL

Since its publication by Hill [1], the use of the Hill equation has been widespread, especially for curve-fitting purposes. In pharmacology, the Hill model has been the basic equation for many PK–PD models describing both static and dynamic effects. This aspect of the Hill equation is a descriptive, pseudo-deterministic one. However, the use of the Hill equation in this way is restricted to its mathematical properties.

Although interesting, the above modelling approach may bring no more than a simple description of a relationship, and may fail to explore the underlying physical or biological mechanisms involved. If the purpose is 'just curve fitting' [53], other equations may also work well, and may be used interchangeably [54].

Hill's work may also be considered as an example of a sensible approach in nonlinear modelling. In 1910, Hill considered both types of model: a descriptive one [Eqn (1)] and a mechanistic one [Eqn (2)] to describe the relationship between his two sets of data. In nonlinear modelling, one should probably follow Hill's example and consider various solutions to describe a relationship whenever possible. Current computational capabilities of numerical calculation should lead us to explore and test various models to represent the same system, including mechanistic/explanatory models, even if they are more complex than a curve-fitting numerical solution.

The Hill equation can be more than a descriptive model. In the setting of a single physicochemical equilibrium, and some specific multiple binding patterns [36], the model shows very interesting explanatory and mechanistic properties, in direct connection with the law of mass action. In the setting of multiple equilibria, Hill's equation B [Eqn (1)] seems to be not only a simpler solution (i.e. making calculation easier) but also a 'heuristic solution' [55]. This equation, with a real valued α coefficient [Eqn (0)], may provide a simplified expression to describe multiple simultaneous equilibria in a single phase, each one of those being characterized by a Hill expression with an integer valued coefficient. In another words, the Hill equation might be considered as a 'compromise equation', extending the law of mass action to multiple simultaneous equilibria, providing a single expression for a hypothetical single 'median equilibrium'.

Another point of interest is the probabilistic view of the Hill equation as it can also describe a cumulative distribution function $F(x)$ of a random variable X ($x \in R^+$). Modelling drug-induced efficacy and toxicity is a very interesting potential application of this approach in pharmacology.

In this review, the various properties of the Hill model have been presented separately for ease of presentation. However, the boundaries between them are often blurred in many settings. Interestingly, in models describing gating transitions of voltage-dependent ion channels, both descriptive and probabilistic properties of the Hill model have been used to analyse the same system. Models featuring gating currents have used the descriptive Hill equation [11,12] while models featuring the open probability of channels have used the probabilistic approach [38,39]. Moreover, a mechanistic interpretation has been assumed in this setting, as the value observed for the Hill coefficient was assumed to represent either the number of ligand binding patterns required, or an index of cooperativity between subunits to get the channel opened. This is another example where a single Hill equation may represent a 'median equilibrium' as discussed above.

Further, in pharmacology, most experimental measurements are not related to a single drug–receptor interaction, but are actually aggregated measures of the effect of a drug on a large set of receptors (which can be

isolated proteins or located on cultured cells, bacterial cells, cancer cells, viruses, etc.). Probably each receptor may interact with the drug with its own sensitivity. The drug sensitivity is variable from one receptor to another and can also be influenced by many factors including interactions involving neighbouring receptors. One might then suppose that some statistical distribution would describe the receptors sensitivity. This theory was proposed in 1924 by Shackell et al. [56] and has still been supported thereafter [13]. Therefore, the adequacy of the Hill model to describe dose – or concentration – effect relationships might lie between the mechanistic model (the physicochemical interaction at the receptor level) and the probabilistic distribution model (the aggregated measured effect).

CONCLUSION

The introduction of the Hill equation in 1910 is truly the beginning of quantitative pharmacology [57]. This three-parameter nonlinear equation has shown many interesting properties and applications in biosciences.

First, the descriptive properties of the Hill model have been widely used to fit a model to experimental or clinical data when the relationship between two sets of variables seems saturable and nonlinear. Second, the mechanistic aspect of the Hill model stems from physicochemical equilibrium principles: the Hill model can describe specific binding patterns, each parameter of the model having a physical meaning in this setting.

Third, the Hill equation may be used as a probabilistic expression. The three-parameter equation represents then a family of cumulative distribution functions. This approach has not been extensively studied and we provide here some new insights into this field. The probability of time-dependent biological events such as adverse reaction, toxicity or death may be described by a probabilistic Hill equation. There are probably many potential applications to such an approach in pharmacology and biology.

Finally, one might conclude that there are not one but several Hill models, each for its own use. However, the boundaries between all the aspects of the Hill equation overlap in many situations, and Hill's historical issue was one of them.

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APPENDIX

A. Descriptive properties of the Hill equation

Expressions of the first and the second derivatives of Eqn (1)

The first derivative is given by:

$$y_1' = 100\frac{K\alpha x^{\alpha-1}}{(1+Kx^{\alpha})^2} \qquad (A1)$$

The second derivative has the following expression:

$$y_1'' = 100\frac{x^{\alpha-2}}{(1+Kx^{\alpha})^3}[(\alpha-1)-Kx^{\alpha}(\alpha+1)] \qquad (A2)$$

B. Probabilistic properties of the Hill equation

Calculation of moments

The expression of the kth-order moment is given by:

$$E(X^k) = \int_0^{\infty} x^k f(x)dx = \int_0^{\infty} x^k \frac{(\alpha/c)(x/c)^{\alpha-1}}{[1+(x/c)^{\alpha}]^2}dx \qquad (A3)$$

The kth-order moment is defined only if $k < \alpha$.

Using the Euler function (gamma function), $\Gamma(x)$, Eqn (A3) is equivalent to [37]:

$$E(X^k) = c^k\Gamma(1+k/\alpha)\Gamma(1-k/\alpha) \qquad (A4)$$

When $\alpha > 1$, the mean (first moment) is defined by [37]:

$$E(X) = c\Gamma(1+1/\alpha)\Gamma(1-1/\alpha) \qquad (A5)$$

When $\alpha > 2$, the variance (second moment) is defined by:

$$\sigma^2 = E(X^2) - E^2(X) = c^2\left[\Gamma\left(1+\frac{2}{\alpha}\right)\Gamma\left(1-\frac{2}{\alpha}\right) - \Gamma^2\left(1+\frac{1}{\alpha}\right)\Gamma^2\left(1-\frac{1}{\alpha}\right)\right] \qquad (A6)$$

Calculation of quantiles

As the expression of the cumulative distribution function is known, one obtains the different quantiles directly. A quantile x_q of a distribution function is the solution of the equation $F(x_q) = q$ (q ranging from 0 to 1). The quantile is single when the cumulative distribution function is continuously increasing, as in this case.

The expression of a quantile for a Hill distribution is given by Eqn (A7):

$$q = \frac{(x_q/c)^{\alpha}}{1+(x_q/c)^{\alpha}} \qquad (A7)$$

the solution of which is:

$$x_q = c\left(\frac{q}{1-q}\right)^{1/\alpha} \qquad (A8)$$

With any two quantiles, x_q and $x_{q'}$, we have:

$$\frac{x_q}{x_{q'}} = \left[\frac{q/(1-q)}{q'/(1-q')}\right]^{1/\alpha} \qquad (A9)$$

In the case of symmetric quantiles x_q and $x_{q'} = x_{1-q}$, as $q + q' = 1$, Eqn (A9) becomes:

$$\frac{x_q}{x_{1-q}} = \left[\frac{q}{1-q}\right]^{2/\alpha} \qquad (A10)$$

and then, using the natural logarithm of each term of Eqn (A10) we have:

$$\alpha = 2\frac{\ln[q/(1-q)]}{\ln[x_q/x_{1-q}]} \qquad (A11)$$

Therefore, we obtain a general relationship between the quantiles and the α parameter of the Hill equation which may permit calculation of the α parameter if the quantiles are known. For example, $\alpha = 2\ln 3/\ln(x_{0.75}/x_{0.25})$ with the quartiles ($q = 0.75$) and $\alpha = 4\ln 3/\ln(x_{0.9}/x_{0.1})$ with the deciles ($q = 0.9$).

Moreover, with symmetric quantiles, a simple relationship also exists between the quantiles and parameter c:

$$c = (x_q x_{1-q})^{1/2} \qquad (A12)$$

Mathematical basis of probit analysis

The probit method has been widely used in biology to analyse quantal (all-or-none) responses. If p is the response proportion related to the value x of a variable X, the probit analysis consists in a transformation of the

response p into probability units (or probits). This transformation is:

$$y = \Phi^{-1}(p) \qquad (A13)$$

where Φ^{-1} is the $100 \times p$ quantile from the standard normal distribution $N(0,1)$. Note that the expression $y' = y + 5$ may be used instead of y in order to avoid negative values.

In a probit analysis, it is assumed that the distribution of the transformed responses y is Gaussian, and therefore

$$Y = \frac{X - m}{\sigma} \qquad (A14)$$

where m and σ are the mean and the standard deviation of the assumed normal distribution, respectively. Eventually, the parameters m and σ can be estimated by linear regression.

Logit expression of the logistic function

Applied to the logistic cumulative distribution function:

$$F(x) = p = P(X \leqslant x) = \frac{1}{1 + \exp[-(x - \mu)/s]}, \qquad (A15)$$

$x \in]-\infty,+\infty[$, $\mu \in R$, $s \in R^+$ where μ (which is the mean) and s are the parameters of the distribution, the logit transformation leads to:

$$\ln\left(\frac{F(x)}{1 - F(x)}\right) = \frac{x - \mu}{s} \qquad (A16)$$

This simple linear relationship allows probably the most convenient application of the GLM:

$$P(A|X_1 = x_1, \ldots, X_n = x_n) = \Phi\left(\sum_{i=1,\ldots,n} a_i x_i\right) \qquad (A17)$$

by using the above logistic cumulative distribution function as the generic function Φ. Thus, one can express the probability P of an event A by a linear development of several variables X_i.

Calculation of Shannon entropy

Shannon entropy is defined by: $I_S = -\int_0^\infty \ln(f(x))f(x)\mathrm{d}x$. For the Hill distribution, the calculation leads to [55]:

$$I_S = 2 + \ln c - \ln \alpha \qquad (A18)$$

Calculation of Fisher information matrix

The Fisher information I_F of a distribution whose density function is f is given by:

$$I_F = -E\frac{\partial^2 \ln f}{\partial \theta^2} \qquad (A19)$$

where θ represents distribution parameters. The information I_F is a scalar if θ is a scalar parameter, and is a square matrix (m,m) if θ is a vector parameter with dimension m.

Applied to a Hill expression, $F(\alpha, c)$, the calculation of Fisher information matrix leads to [55]:

$$\begin{aligned} I_F &= -\begin{pmatrix} E(\partial^2 \ln f/\partial c^2) & E(\partial^2 \ln f/\partial c \partial \alpha) \\ E(\partial^2 \ln f/\partial c \partial \alpha) & E(\partial^2 \ln f/\partial \alpha^2 \end{pmatrix} \\ &= \begin{pmatrix} \frac{\alpha}{3c^2} & 0 \\ 0 & \frac{\pi^2+3}{9\alpha^2} \end{pmatrix} \end{aligned} \qquad (A20)$$

and also

$$V = \frac{1}{n} I_F^{-1} = \frac{1}{n}\begin{pmatrix} \frac{3c^2}{\alpha} & 0 \\ 0 & \frac{9\alpha^2}{\pi^2+3} \end{pmatrix} \qquad (A21)$$

where V is the variance–covariance matrix of the maximum likelihood estimators of the parameters c and α for a sample of size n.