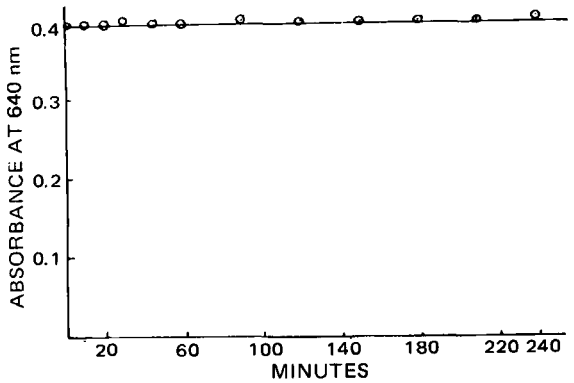

Figure 5—*Stability of colored product.*

For the present study, tablet samples were obtained from the local market. Five out of the eight samples were found by TLC to contain lactose and lactose isonicotinoyl hydrazone. Lactose-containing tablets (Table III, Samples A–D), when assayed by the proposed procedure, were found to contain between 78 and 90% of the labeled amount of free isoniazid. As much as 10–22% of isoniazid was present in the bound form with lactose and was probably not available for absorption. On the other hand, quantitative recoveries were obtained by the official method in the analysis of these tablets. Tablets that did not contain lactose gave comparable recoveries by both the official and the proposed methods.

When freshly prepared lactose-containing granules and the tablets prepared from them (Table III, Samples E and F) were analyzed by the proposed method, interaction between isoniazid and lactose was only 1–3%. Therefore, the significant interaction between isoniazid and lactose in the tablets apparently occurs only on standing over an extended period. The interaction seems to be negligible at the granulation stage.

The proposed method is specific for the estimation of isoniazid in the presence of its hydrazones. Significant interaction between isoniazid and lactose, resulting in the formation of lactose isonicotinoyl hydrazone, has been established. This interaction is likely to interfere with the bioavailability of isoniazid from its dosage forms.

Table III—Analysis of Isoniazid Tablets

Sample	Labeled Amount, mg/tablet	Recovery[a], mg/tablet: USP Method	Recovery[a], mg/tablet: Proposed Method	Presence of: Isoniazid Hydrazone	Presence of: Lactose
A	100	98.60	86.83	+	+
B	50	50.73	39.50	+	+
C	50	51.94	39.17	+	+
D	100	100.90	89.72	+	+
E[b]	300	305.21	301.20	+	+
F[c]	300	306.97	297.76	+	+
G	100	99.70	99.52	−	−
H	300	308.83	304.92	−	−
I	300	297.80	299.65	−	−

[a] Average result of three determinations. [b] Freshly prepared tablets. [c] Granules ready for the preparation of tablets.

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Mathematical Basis of Point–Area Deconvolution Method for Determining *In Vivo* Input Functions

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Abstract □ The point–area method for deconvolution derives a "staircase" input function which, when convolved onto the characteristic function, gives an output function coincidental with the given output data points. The area–area method for deconvolution is shown to be erroneous.

Keyphrases □ Deconvolution—point–area and area–area methods for determining *in vivo* input functions compared □ Input functions, *in vivo*—deconvolution point–area and area–area methods of determination compared □ Pharmacokinetics—deconvolution point–area and area–area methods for determining *in vivo* input functions compared

The use of *in vitro* dissolution functions for predicting differences in the rate and extent of *in vivo* drug dissolution depends on a correspondence (isomorphism) between the two processes. Any rigorous investigation of such an isomorphism ultimately requires the derivation of the *in vivo* drug input function. This function can be derived for

linear systems by mathematical or numerical deconvolution of the response to some particular drug input and the characteristic response of the system (1–6).

A known method for numerical deconvolution is the so-called area–area method as detailed by Rescigno and Segre (7). However, this method is ambiguous, if not erroneous. Furthermore, there seems to be some confusion about its exact mathematical basis. For example, Benet and Chiang (8) implied that the method is only appropriate when the characteristic response is a single exponential function, and Wagner (9) shifted the derived data to recover a known input function, which would be inappropriate for an arbitrary input function.

For clarification, this deconvolution method is now derived, and it is shown how it relates an unknown input function to a "staircase" input.

THEORY AND DISCUSSION

If the body is regarded as a linear system, then the blood drug concentration–time curve, $Y(t)$, obtained with an arbitrary but finite drug input into the body can be described by the convolution integral:

$$Y(t) = \int_0^t \ln(\tau)G(t-\tau)\,d\tau = \ln(t)*G(t) \qquad \text{(Eq. 1)}$$

where $G(\tau)$ is the blood drug concentration–time function obtained with a unit drug impulse input (*i.e.*, the characteristic response) and $\ln(\tau)$ is the function that, when integrated between limits of $t = 0$ and t, yields the cumulative amount of drug delivered to the impulse input point [*i.e.*, $\ln(\tau)$ is the input function]. Throughout, it is assumed that both $G(t)$ and $\ln(t)$ are bounded nonnegative functions and Riemann integrable on $[0, \infty]$.

Even if $G(\tau)$ and $Y(\tau)$ are known analytically, the integral function defined by Eq. 1 cannot be solved, except in certain cases, for $\ln(\tau)$ by general formulas because the Laplace transform of Eq. 1:

$$y(s) = \ln(s)g(s) \qquad \text{(Eq. 2)}$$

with solution:

$$\ln(s) = \frac{y(s)}{g(s)} \qquad \text{(Eq. 3)}$$

cannot be transformed back into time space by the convolution theorem since $1/g(s)$ is not a Laplace transform (10). This fact indicates the need for general deconvolution methods. In general, numerical evaluation of $y(s)$ and $g(s)$, with subsequent numerical calculation of $\ln(s)$ and inversion, is unsuccessful because of instability (11, 12). Fourier transform methods have been used and are reasonably accurate (12). However, a simple numerical method would be advantageous.

Response to Staircase Input—A staircase input is defined as a finite set of rectangular pulses of duration $p_j - p_{j-1}$ and intensity I_j, each commencing at $t = p_{j-1}$ and ending at $t = p_j$ ($j \in 1, 2, 3 \ldots$), where $I_j \geq 0$, $t \geq 0$, and $p_0 = 0$. Then the response of a linear system to a staircase input at the time points p_j can be obtained by application of standard Laplace transformation methods (10):

$$Y(p_j) = \sum_{i=1}^{j} I_i \left[\int_0^{p_j - p_{i-1}} G(\tau)\,d\tau - \int_0^{p_j - p_i} G(\tau)\,d\tau \right] \qquad \text{(Eq. 4)}$$

where $j \in 1, 2, 3 \ldots$, $p_0 = 0$, and $Y(0) = 0$.

In particular, if the staircase pulse lengths are all equal, say $p_j - p_{j-1} = a$ for all j, then substitution of $p_j = ja$ and $p_i = ia$ into Eq. 4 gives:

$$Y(ja) = \sum_{i=1}^{j} I_i \int_{(j-i)a}^{(j-i+1)a} G(\tau)\,d\tau \qquad \text{(Eq. 5)}$$

where $j \in 1, 2, 3 \ldots$ and $Y(0) = 0$.

Given the exact output, $Y(p_j)$ at time points p_j or $Y(ja)$ at time points ja, and the function $G(\tau)$, a particular staircase input function can be recovered exactly by algebraic manipulation of Eq. 4 or 5. The details for an equal pulse length staircase input function are as follows.

By defining the pulse length as a and:

$$\int_{(n-1)a}^{na} G(\tau)\,d\tau \equiv A_n \qquad \text{(Eq. 6)}$$

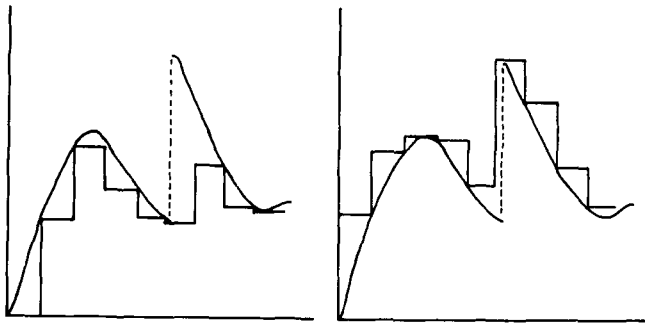

Figure 1—*Representation of an input function by a minimum (left) and maximum (right) staircase function.*

where $n \in 1, 2, 3 \ldots$, and then by applying Eq. 5 and rearranging, the I's are given by:

$$I_1 = \frac{Y(a)}{A_1} \qquad \text{(Eq. 7a)}$$

$$I_2 = \frac{Y(2a) - I_1A_2}{A_1} \qquad \text{(Eq. 7b)}$$

$$I_3 = \frac{Y(3a) - I_1A_3 - I_2A_2}{A_1} \qquad \text{(Eq. 7c)}$$

$$\vdots$$

$$I_n = \frac{Y(na) - I_1A_n - I_2A_{n-1} \ldots I_{n-1}A_2}{A_1} \qquad \text{(Eq. 7d)}$$

A similar set of equations can be constructed for unequal pulse lengths by applying Eq. 4.

Deconvolution Using Staircase Functions—Given the output of a linear system, $Y(p_j)$, at time points $t = p_j$ corresponding to some arbitrary input function, $\ln(t)$, and the appropriate $G(\tau)$, a staircase input function can always be derived from the data by applying Eq. 4 or 5. This derived staircase input function, $U_s(t)$, when convolved on $G(\tau)$, will yield a function exactly coincidental with the given output data points $Y(p_j)$ at $t = p_j$. Since $U_s(t)$ is derived using specific output data points and the integral of $G(\tau)$, it is appropriate to designate this deconvolution method as the point–area method.

That each step of the derived staircase function, $U_s(t)$, intersects the true input function, $\ln(t)$, can be shown as follows. For any nonnegative bounded and finite drug input, a staircase function, $U_1(t)$, exists such that the magnitude of each step is equivalent to the minimum value of $\ln(t)$ in each step period (Fig. 1, left), since:

$$\int_0^t U_1(\tau)\,d\tau < \int_0^t \ln(\tau)\,d\tau \qquad \text{(Eq. 8)}$$

the convolution of $U_1(t)$ on $G(\tau)$ is always less than the true output function in the time interval $(0, \infty)$. Similarly, there exists a staircase function, $U_2(t)$, such that the magnitude of each step is equivalent to the maximum value of $\ln(t)$ in each step period (Fig. 1, right), since:

$$\int_0^t U_2(\tau)\,d\tau > \int_0^t \ln(\tau)\,d\tau \qquad \text{(Eq. 9)}$$

the convolution of $U_2(t)$ on $G(\tau)$ is always greater than the true output function in the time interval $(0, \infty)$. Consequently, the staircase function, $U_s(t)$, which, when convolved on $G(\tau)$ is coincidental with the true output

Table I—Comparison of the Cumulative Drug Input and That Determined by Deconvolution [a]

Hours	Exact Cumulative Input, %	Estimated Cumulative Input, %	Error, %
1	49.99	49.15	−1.68
2	74.99	73.75	−1.65
3	87.49	86.07	−1.63
4	93.75	92.24	−1.61
5	96.87	95.33	−1.59
6	98.44	96.89	−1.57
7	99.22	97.67	−1.56

[a] Output data $Y(t)$ were generated at the times shown by application of Eq. 1, where $G(t) = 5e^{-0.4t} + 5e^{-0.2t}$ and $\ln(t) = 0.693e^{-0.693t}$. Cumulative drug input was estimated by application of Eqs. 7a–7d, and the exact input was determined by the integration of the input function.

Table II—Maximum Errors Associated with the Deconvolution for Various Input Functions [a]

Input Function α	Maximum Error, %
2	−4.56
1.5	−3.52
1.0	−2.40
0.693	−1.68
0.35	−0.86

[a] Output data $Y(t)$ were generated at $t = 1$ by Eq. 1 using the same $G(t)$ as in Table I and various input functions, $\ln(t) = \alpha e^{-\alpha t}$. Cumulative drug input was estimated by application of Eq. 7*a*, and the exact input was determined by the integration of the input function.

at the end of each step, intersects or is coincidental with the true input function in the period of each step.

The exact points of intersection for $U_s(t)$ and $\ln(t)$ cannot be specified since they depend on the exact form of the unknown input function. However, the cumulative *in vivo* drug input is the function most often required for comparison with *in vitro* dissolution data. The cumulative *in vivo* input is readily obtainable by multiplication of the derived I values by the period of each step and summation.

Since the derived staircase input function, $U_s(t)$, does not depend on the form of $G(\tau)$, the method is equally appropriate for any $G(\tau)$. Obviously, the accuracy of the derived cumulative input depends on the step length since, as all step lengths tend to zero, $U_s(t) \rightarrow \ln(t)$.

The cumulative input derived by the point–area method is an excellent approximation of the true cumulative input. Some results are presented in Table I. These data assume an exponential input function and a sampling period equal to the half-life of the input function; such an input function and sampling period are likely to represent extremes of those occurring in practice. The disposition function, $G(\tau)$, was arbitrarily chosen as a biexponential function.

As indicated by the data in Table I, the derived cumulative input slowly converges onto the true cumulative input and the maximum error occurs in the first period. The latter error is a function of the input function and the first output data point, as illustrated by the data in Table II. The 4.56% error corresponding to $\alpha = 2$ represents an extreme case since 86% of the input occurs in the first period.

Erroneous Area–Area Method of Deconvolution—Rescigno and Segre (7) described a perfectly valid method for the numerical convolution of two functions. Essentially, this method approximates one function to an equal pulse length staircase function such that the area of each step is identical to the area of the function itself during each step. The corresponding areas of the staircase function are then convolved on the other function. Analytically, this method corresponds to the multiplication of Eq. 5 by the pulse lengths, say a, and the output is given as $aY(ja)$, $j \in 1, 2, 3 \ldots$. Unfortunately, when inversing the procedure (*i.e.*, deconvolution), $aY(ja)$ is interpreted as the actual area of the output function:

$$\int_{(j-1)a}^{ja} Y(t)\,dt = aY(ja) \qquad \text{(Eq. 10)}$$

Obviously, the equality of Eq. 10 is erroneous for most output functions, $Y(t)$. Nevertheless, this method of deconvolution has been used and designated as the area–area method of deconvolution (8).

A comparison of the percentage errors of the area–area and point–area methods is given in Fig. 2. Output data $Y(t)$ were generated by Eq. 1 where $G(t) = 5e^{-0.2t}$ and $\ln(t) = 0.5e^{-0.5t}$. Exact cumulative input was determined by integration of the input function. The estimated cumulative input was determined by the method of Rescigno and Segre (7) for the area–area method and by Eqs. 7*a*–7*d* for the point–area method. As expected, the area–area method gave large and generally unpredictable errors.

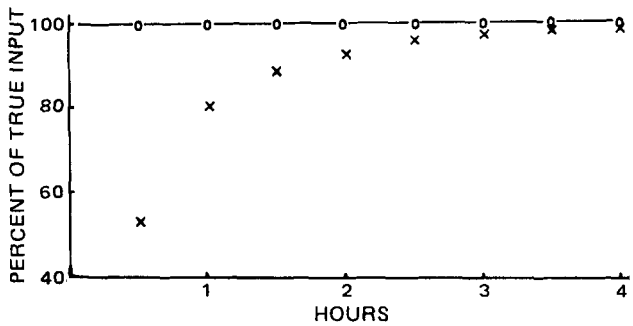

Figure 2—*Percentage errors in the cumulative drug input obtained by the point–area (O) and the area–area (×) methods of deconvolution. The solid line represents zero error.*

Input functions, as used in Eq. 1, define the drug input to the point at which an impulse input is applied to the body. The actual physical meaning of this function depends on how the characteristic response is defined. For example, if the characteristic response is defined as the resulting blood concentration–time function after an intravenous bolus dose and the data after an oral solution dose are deconvoluted, the resulting input function represents both drug absorption and transport through the liver. These concepts and definitions of input functions were discussed previously (13).

In conclusion, deconvolution using a staircase input function is a simple and accurate method for the assessment of cumulative *in vivo* drug input. The method does not require equally spaced output data points either during or after drug absorption as suggested by Wagner (9).

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