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The Empirical Evaluation of the Initial Velocities of Enzyme-catalyzed Reactions[1]

BY KEITH A. BOOMAN[2] AND CARL NIEMANN[3]

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A practical and objective procedure for the empirical evaluation of the initial velocities of enzyme-catalyzed reactions has been developed and has been applied in a number of cases where alternative procedures based upon assumed rate equations could lead to biased results. The new procedure is based upon fitting a set of orthogonal polynomials to the data of a particular experiment by the method of least squares, employing the "t" test to determine the significance of the coefficients of each of the polynomials relative to the evaluated experimental error and thus to select a suitable polynomial for the representation of the experimental data, transforming the selected polynomial into a series of the form $C_0' + C_1'(s) + C_2'(s)(s-1) + \ldots$, differentiating the above series to arrive at a value of the initial velocity and finally determining the variability of the latter value.

For the past four years we have been concerned with the development of procedures for the objective evaluation of the initial velocities of enzyme-catalyzed reactions in those cases where a rate equation can be assumed on the basis of prior knowledge or is established in the course of the evaluation procedure.[4-9] However, if the reaction being studied cannot be described in terms of a relatively simple rate equation and this fact is not revealed by the evaluation procedure, or alternatively if it is revealed but the information at hand does not permit the development of a satisfactory rate equation, it is clear that the forced application of any of the above procedures[4-9] may lead to biased values of the initial velocities. Since we have encountered a number of situations where knowledge of the initial velocities was desirable but where the available data were not sufficiently extensive to permit the development of satisfactory rate equations, we decided to direct our attention to the development of an empirical procedure for the evaluation of the initial velocities of enzyme-catalyzed reactions, which by definition does not require any information relative to the rate equation of the reaction under investigation, rather than to accept the risk of obtaining biased values of the initial velocities in these particular situations by the forced application of procedures based upon specific rate equations.[4-9]

The procedure which has been developed is based upon fitting a set of orthogonal polynomials to the data of a particular experiment by the method of least squares[10] and employing the "t" test[11] to determine the significance of the coefficients of each of the polynomials relative to the evaluated experimental error in order to select a suitable polynomial for the representation of the experimental data. The selected polynomial is transformed into a series of the form $C_0 + C_1'(s) + C_2'(s)(s-1) + \ldots$ and this series is then differentiated to arrive at a value of the initial velocity. Finally the variability of this latter value is determined. The only limitation of this general procedure is that the observations provide for a point corresponding to time equal zero and that the experimental observations with respect to the extent of reaction be made at equal time intervals or that such information be obtained from the actual observations by an unequivocal interpolation procedure.

In order to facilitate use of the above procedure, it has been set up in a block form which will permit the necessary computations to be made with a desk calculator in about 20 minutes. With the aid of the tables of orthogonal polynomials for $n + 1$ equally spaced points which are given in Milne,[12] the procedure may be employed for experiments involving from six to twenty-one points. It will be noted that the highest order polynomial considered in reference 10 is $P_{5,n}$. However, this is not a limitation since it has been found that even $P_{4,n}$ is rarely required.

In this communication we shall consider the development of the procedure for the evaluation of an experiment involving eight points, including the one corresponding to zero time, and shall give the necessary constants for the evaluation of a nine point experiment.[13] The notation of Milne[10] and of Bennett and Franklin[11] will be used throughout.

a. **Evaluation of the Coefficients $C_{m,n}$ of the Tabulated Orthogonal Polynomials for $n = 7$.**—For the case where $n = 7$, *i.e.*, for an experiment with eight points including the one corresponding to zero time, the first step is to evaluate the coefficients $C_{m,n}$ of the tabulated orthogonal polynomials selected for the representation of the experiment. $C_{m,n}$ are the coefficients of the tabulated orthogonal polynomials listed by Milne[12] and are the constants determined in line C of the example given by the same authors.[14] The polynomials with which we will be concerned are

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(2) U. S. Rubber Co. Fellow.

(3) To whom inquiries regarding this article should be sent.

(4) R. R. Jennings and C. Niemann, THIS JOURNAL, **75**, 4687 (1953).

(5) R. J. Foster and C. Niemann, *Proc. Natl. Acad. Sci.*, **39**, 999 (1953).

(6) T. H. Applewhite and C. Niemann, THIS JOURNAL, **77**, 4923 (1955).

(7) R. R. Jennings and C. Niemann, *ibid.*, **77**, 5432 (1955).

(8) K. A. Booman and C. Niemann, *ibid.*, **77**, 5733 (1955).

(9) W. E. M. Lands and C. Niemann, *ibid.*, **77**, 6508 (1955).

(10) W. E. Milne, "Numerical Calculus," Princeton University Press, Princeton, N. J., 1949.

(11) C. A. Bennett and N. L. Franklin, "Statistical Analysis in Chemistry and the Chemical Industry," John Wiley and Sons, New York, N. Y., 1954.

(12) Reference 10, pp. 375–381, Table VI.

(13) Eight and nine point runs have been selected because of their pertinence to experiments conducted in 10-ml. volumetric flasks and involving the withdrawal of 1-ml. aliquots at a selected time interval.

(14) Reference 10, pp. 271–273.

$$P_{0.7}(s) = 1$$

$$P_{1,7}(s) = 7 - 2(s)$$

$$P_{2,7}(s) = 7 - 16(s) + 1(s)(s - 1)$$

$$P_{3,7}(s) = 7 - 12(s) + 6(s)(s - 1) - 0.6666(s)(s - 1)(s - 2)$$

$$P_{4.7}(s) = 7 - 20(s) + 15(s)(s - 1) - 4.6666(s)(s - 1)(s - 2) + 0.5833(s)(s - 1)(s - 2)(s - 3)$$

$$P_{5.7}(s) = 7 - 30(s) + 35(s)(s - 1) - 18.6666(s)(s - 1)(s - 2) + 5.25(s)(s - 1)(s - 2)(s - 3) - 0.7(s)(s - 1)(s - 2)(s - 3)(s - 4)$$

These polynomials, which are those whose values are given by Milne[12] for $n = 7$, were obtained from the general polynomials given by Milne,[15] except that each polynomial above $P_{0,7}(s)$ has been multiplied by the number at the top of its column in the table[12] for $n = 7$, *i. e.*, in each instance by 7 except for the one noted above.

b. **Test for Significance of Each Coefficient $C_{m,n}$.**—The question of what order polynomial should be used is determined on the basis of the "t" test for significance[11] by considering the significance of each coefficient $C_{m,n}$ as it is evaluated in step a above. Since, in the case at hand, $t_{(n-m)}$ is the ratio of $C_{m,7}$ to its standard deviation, we shall regard a coefficient $C_{m,7}$ as significant if $t_{(n-m)}$ is larger than a prescribed value. As in the example considered in Bennett and Franklin,[16] $t_{(n-m)}$ is computed on the basis of equations 1 and 2 where S_m is defined as in Milne[12]

$$t_{(n-m)} = (S_m)^{1/2} C_{m,n}/\sigma_{y.m} \tag{1}$$

$$\sigma_{y.m} = \left\{\sum_{i=0}^{n} (y_i)^2 - (n + 1(\bar{y})^2) - \sum_{k=1}^{m} S_k(C_{k.n})^2/(n - m)\right\}^{1/2} \tag{2}$$

$\sigma_{y,m}$ is the standard deviation of the experimental data calculated on the basis of an orthogonal polynomial representation of degree m,y_i is the value of the dependent variable and $\bar{y}$ is the arithmetic average of the values of y_i. It will be noted that the quantity $(n - m)$ is equal to the number of degrees of freedom and also is equal to the number of points in the run, *i. e.*, $(n + 1)$, minus the number of coefficients to be determined for the polynomial, *i. e.*, $(m + 1)$. In practice values of $t_{(n-m)}$ computed on the basis of equations 1 and 2 are compared with values of $t_{(n-m),\alpha}$ which are given in a table of the percentage points of the Student "t" distribution.[17] In our studies α has been taken as either 0.05 or 0.10. If $t_{(n-m)}$ is greater than $t_{(n-m),0.05}$ the risk in accepting $C_{m,n}$ as actually being different from zero is less than 5%.

A special case arises where sufficient information is available, *e.g.*, through repeated observation of the characteristics of a given system, to recognize that the true value of $C_{m,n}$ is either positive or negative. In this instance instead of testing whether the coefficient is significantly different from zero, one immediately rejects constants which have the wrong sign and then tests the remaining coefficients to determine whether they are significantly greater than zero.[18] In this case $t_{(n-m)}$ is compared with $t_{(n-m),2\alpha}$ if the risk in accepting $C_{m,n}$ as being different from zero is to be less than α.

c. **Transformation of the Orthogonal Polynomial Representation into a Series Representation.**—In order to facilitate subsequent differentiation, it is convenient to transform the orthogonal polynomial representation into a series representation of the form given by equation 3. It is evident from equation 3 that C_m' is obtained by summing

$$\bar{y} = C_0' + C_1'(s) + C_2'(s)(s - 1) + \ldots \tag{3}$$

$$C_0' = C_{0.7} + 7C_{1.7} + 7C_{2,7} + \ldots$$

$$C_1' = -2C_{1.7} + 6C_{2.7} + 12C_{3,7} + 20C_{4,7} + 30C_{5,7}$$

$$C_2' = C_{2.7} + 5C_{3,7} + 15C_{4,7} + 35C_{5.7}$$

$$C_3' = -0.6666C_{3,7} + 4.6666C_{4.7} + 18.6666C_{5,7}$$

$$C_4' = 0.5833C_{4,7} + 5.25C_{5,7}$$

$$C_5' = -0.7C_{5,7}$$

the contribution of the m^{th} power terms in each polynomial to the final answer. Thus, the coefficient of $(s)(s - 1)$ is determined by first noting that it occurs in $P_{2,7}$, $P_{3,7}$, $P_{4,7}$ and $P_{5,7}$. The contribution from $P_{2,7}$ is $1C_{2,7}$, the contribution from $P_{3,7}$ is $6C_{3,7}$, etc.

d. **Differentiation of Series Representation and Evaluation of the Initial Velocity.**—Differentiation of equation 3 leads to equation 4. It is seen from

$$\mathrm{d}\bar{y}/\mathrm{d}s = C_1' + \mathrm{C}'(2s - 1) + C'(3s^2 - 6s + 2) + C'(4s^3 - 18s^2 + 22s - 6) + C_5'(5s^4 - 40s^3 - 105s^2 - 100s + 24) \tag{4}$$

equation 4 that the quantity $(\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0}$ is given by equation 5 and that substituting for values of

$$(\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0} = 1C' - 1C_2' + 2C_3' - 6C' + 24C' \tag{5}$$

C_m' will lead to equation 6. We can now evaluate

$$-(\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0} = (2C_{1.7} + 6\mathrm{C}_{2,7} + 12C_{3.7} + 20C_{4.7} + 30C_{5,7}) + (C_{2.7} + 5C_{3.7} + 15C_{4,7} + 35C_{5.7}) + 2(0.6666C_{3.7} + 4.6666C_{4.7} + 18.6666C_{5,7}) - 6(0.5833C_{4,7} + 5.25C_{5.7}) + 24(0.7C_{5,7}) \tag{6}$$

$(\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0}$ for any order polynomial up to and including the fifth order by collecting terms in equation 6 to give equation 7. If, for example, a

$$-(\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0} = 2C_{1,7} + 7\mathrm{C}_{2.7} + 18.333C_{3,7} + 47.833C_{4,7} + 150.633C_{5.7} \tag{7}$$

third-order polynomial is selected on the basis of considerations outlined in step b above, only the first three terms of equation 7 are calculated. The initial velocity, *i. e.*, v_0, is then evaluated from the relationship given in equation 8 where $\Delta\tau$ is the

$$v_0 = -(\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0}/b\Delta\tau \tag{8}$$

time interval at which the observations were made and b is a factor which is used to convert values of y to values of concentration.

e. **Determination of the Variability of the Value of v_0.**—It has been shown[14] that the quantity C_m is given by equation 9 from which we may derive equation 10. Since v_0 is calculated from a

$$C_m = \left(\sum_{s=0}^{n} P_{m.n}(s)f(s)\right)/S_m \tag{9}$$

$$\sigma_{C_m} = \sigma_y/(S_m)^{1/2} \tag{10}$$

linear combination of values of C_m, we may evaluate σ_{v_0}, for $n = 7$, on the basis of equation 11, which may be transformed into equations 12 and 13.

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(15) Reference 10, pp. 267-268.

(16) Reference 11, p. 258.

(17) Reference 11, p. 696.

(18) Reference 11, p. 139.

$$\sigma_{v_0} = (1/b\Delta\tau)(2^2\sigma^2{}_{C_{1,7}} + 7^2\sigma^2{}_{C_{2,7}} + \cdots + 150.633^2\sigma^2{}_{C_{5,7}})^{1/2} \qquad (11)$$

$$\sigma_{v_0} = (\sigma_y/b\Delta\tau)(2^2/168 + 7^2/168 + \cdots + 150.633^2/2184)^{1/2} \qquad (12)$$

$$\sigma_{v_0} = \sigma_y L_{m,n}/b\Delta\tau \qquad (13)$$

The parameter $L_{m,n}$ in equation 13, which is the quantity given by the square root term of equation 12, is computed but once and values of $L_{m,7}$ and $L_{m,8}$ for values of m up to and including $m = 5$ are given in Table I. It will be seen from the above discussion that if σ for v_0 given by a third-order set of orthogonal polynomials is to be computed, the square root of the first three terms under the square root sign of equation 12, *i. e.*, the value of $L_{3,7}$ given in Table I, is multiplied by the quantity $\sigma_y/b\Delta\tau$.

TABLE I

VALUES OF $L_{m,n}$ FOR $n = 7$ AND $n = 8$

m	1	2	3	4	5
$L_{m,7}$	0.1542	1.139	1.605	2.510	4.100
$L_{m,8}$	0.1291	0.4543	1.061	1.940	2.876

f. Application of the Evaluation Procedure.—In practice it has been found desirable to present the entire evaluation procedure in block form in order to facilitate computation. The form used in these laboratories is illustrated by the following example.

TABLE II

ANALYSIS OF K. A. BOOMAN RUN NO. 34

s	x_i (min.)	y_i (OD$_i$)	$P_0(s)$	$P_1(s)$	$P_2(s)$	$P_3(s)$	$P_4(s)$	$P_5(s)$
0	0	2.140	1	7	7	7	7	7
1	4	1.656	1	5	1	−5	−13	−23
2	8	1.291	1	3	−3	−7	− 3	17
3	12	0.960	1	1	−5	−3	9	15
4	16	.724	1	−1	−5	3	9	−15
5	20	.614	1	−3	−3	7	− 3	−17
6	24	.482	1	−5	1	5	−13	23
7	28	.353	1	−7	7	−7	7	− 7
(A) $S_m = \sum_{s=0}^{7} (P_m(s))^2$			8	168	168	264	616	2184
(B) $c_m = \sum_{s=0}^{7} P_m(s)f(s)$			8.220	20.646	5.454	1.192	− 0.902	0.556
(C) $C_m = (B)/(A)$			1.027 5	1.228 928 × 10⁻¹	3.246 430 × 10⁻²	4.515 152 × 10⁻³	− 1.464 285 × 10⁻³	2.545 790 × 10⁻⁴
(D) $(C_m)^2$			1.055 756	1.510 264 × 10⁻²	1.053 931 × 10⁻³	2.038 660 × 10⁻⁵	2.144 130 × 10⁻⁶	6.481 050 × 10⁻⁸
(E) $\sum_{i=0}^{7} (y_i - \bar{y}_i)^2 = \sum_{i=0}^{7} (\mathrm{OD}_i)^2 - \sum_{k=0}^{m} S_k(C_k)^2 = 11.168322$			2.722 274	1.8503 × 10⁻¹	7.9970 × 10⁻³	2.5873 × 10⁻³	1.2690 × 10⁻³	1.1273 × 10⁻³
(F) $(n - m) = (7 - m)$			7	6	5	4	3	2
(G) $(\sigma_y)^2 = (E)/(F)$				3.0838 × 10⁻²	1.5994 × 10⁻³	6.4682 × 10⁻⁴	4.2301 × 10⁻⁴	5.6367 × 10⁻⁴
(H) σ_y				175.60 × 10⁻³	39.99 × 10⁻³	25.43 × 10⁻³	20.57 × 10⁻³	
(I) $(S_m)^{1/2}$				12.96	12.96	16.25	24.82	46.73
(J) $t_{(n-m)} = (C)(I)/(H)$				9.070	10.520	2.888	1.762	
(K) $t_{(n-m),\,0.05}$					2.571	2.776	3.182	4.303
(L) m				1	2	3	4	5
(M) (Coeff. v_0)				2.000	7.000	18.333	47.833	150.633
(N) $(-\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0} = \sum_{k=1}^{m} C_k\,(\text{coeff. } v_0)_k$				0.2458	0.4730	0.5558		
(O) $v_0(M/\text{min.} \times 10^5) = (N)/b\Delta\tau$						14.20 × 10⁻⁴		
(P) L_m				0.1542	1.139	1.605	2.510	4.100
(Q) $\sigma_{(-\mathrm{d}\bar{y}/\mathrm{d}s)_{s=0}} = L_m\sigma_y$						0.04082		
(R) $\sigma_{v_0}(M/\text{min.} \times 10^5) = (Q)/b\Delta\tau$						1.04 × 10⁻⁴		

The α-chymotrypsin-catalyzed hydrolysis of L-tryptophanhydroxamide was studied in aqueous solutions at 25° and *p*H 6.93 and 0.3 M in the THAM[19] component of a THAM–HCl buffer under conditions where [E] = 0.914 mg. protein-nitrogen per ml. of an Armour preparation No. 00592 and $[S]_0 = 21.79 \times 10^{-3}$ M. One-ml. aliquots were withdrawn from the reaction mixture at 4-minute intervals and the L-tryptophanhydroxamide present in these samples was determined spectrophotometrically essentially by the same pro-

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[19] Tris-(hydroxymethyl)-aminomethane

cedure that was used by Foster, Jennings and Niemann[20] for the determination of L-tyrosinhydroxamide. The primary experimental data so obtained, *i. e.*, optical density, *i. e.*, OD_t, as a function of time, *i. e.*, τ in minutes, are given in Table II.

Table II is so organized that the part of the table above the first dashed line is devoted to the evaluation of the coefficients $C_{m,n}$ of the orthogonal polynomials tabulated for $n = 7$ and $m = 0$ to 5, *cf.*, section *a* above. The values of $P_0(s)$, $P_1(s)$, etc., are those given by Milne.[12] The values of S_m in line (A) are the accumulative squares of the coefficients $P_0(s)$, $P_1(s)$, etc. The values of c_m in line (B) are the results of the accumulative multiplication of the values of OD_i by the corresponding coefficients, *i. e.*, the values of $P_0(s)$, $P_1(s)$, etc. The values of C_m in line (C) are the quotients c_m/S_m, *i. e.*, the quotients of each value in line (B) divided by the corresponding value in line (A) and are the desired coefficients $C_{m,7}$ of the orthogonal polynomials.

The second part of Table II, *i. e.*, the part between the first and second dashed lines, is devoted to computations required for the "t" test, *cf.* section b above. The values of $(C_m)^2$ in line (D) are computed from the values of C_m in line (C) above. In line (E) the quantity $\sum_{i=0}^{7} (\mathrm{OD}_i)^2 - \sum_{k=0}^{m} S_k(C_k)^2$ is evaluated by first computing the accumulative squares of OD_i, entering this value in the OD_i column of line (E) and then successively subtracting from this quantity the accumulative products represented by $\sum_{k=0}^{m} S_k(C_k)^2$ and computed from the values of S_m given in line (A) and the values of $(C_m)^2$ in line (D). The values of $(n - m)$, in this instance $(7 - m)$, are entered in line (F) and in line (G) the quantity $(\sigma_{\mathrm{OD}})^2$ is evaluated as the quotient $(\sum_{i=0}^{7} (\mathrm{OD}_i)^2 - \sum_{k=0}^{m} S_k(C_k)^2/(n - m)$ of the values of the numerator given in line (E) and the corresponding values of the denominator given in line (F). From the values of $(\sigma_{\mathrm{OD}})^2$ in line (G) the corresponding values of σ_{OD} are obtained and entered in line (H). The value of σ_{OD} in any particular column is the estimate of the σ of the observed values of OD based upon the residuals between the approximating polynomial and the primary data. Values of $(S_m)^{1/2}$, in this instance of $(S_{m,7})^{1/2}$, which may be obtained from Table III, are entered in line (I) and in line (J) the quantity $t_{(n - m)}$ is evaluated from the relation $(S_m)^{1/2}C_m/\sigma_{\mathrm{OD}}$ by dividing the product of the values given in lines (C) and (I) of any particular column by the corresponding value given in line (H). The individual values of $t_{(n - m)}$ in line (J) are now compared with the corresponding values of $t_{(n - m),\alpha}$ which are given by Bennett and Franklin[17] for a value of $\alpha = 0.05$, *i. e.*, for an assumed risk of 5%, and which are entered in line (K). It will be seen from the values of $t_{(n - m)}$ and $t_{(n - m),\alpha}$ given in Table II that the values of $t_{(n - m)}$ in columns $P_2(s)$ and $P_3(s)$ are greater than the corresponding values of $t_{(n - m),\alpha}$ and that the value of $t_{(n - m)}$ in column $P_4(s)$ is less than the corresponding value of $t_{(n - m),\alpha}$. Therefore, for the example at hand we shall represent the experimental data by the polynomials $P_0(s)$, $P_1(s)$, $P_2(s)$ and $P_3(s)$ and reject those of higher order.

TABLE III

VALUES OF $(S_{m.n})^{1/2}$ FOR $n = 7$ AND $n = 8$

m	1	2	3	4	5
$(S_{m,7})^{1/2}$	12.96	12.96	16.25	24.82	46.73
$(S_{m.8})^{1/2}$	7.75	52.65	31.46	44.74	21.63

The third part of Table II, *i. e.*, between the second and third dashed lines, relates to the transformation of the orthogonal polynomial representation into a series representation and the subsequent evaluation of the initial velocity, *cf.*, sections c and d above. In line (L) of Table II are the values of m and in line (M) the coefficients for v_0 given by equation 7 and tabulated for values of $n = 7$ and $n = 8$ in Table IV. The quantity $(-\mathrm{d}\bar{y}/\mathrm{d}s)_{s = 0}$ is then computed from the relation $(-\mathrm{d}\bar{y}/\mathrm{d}s)_{s = 0} = \sum_{k=0}^{m} C_k$ (coeff. $v_0)_{m,n}$, in this case by the accumulative multiplication of the values given in lines (C) and (M), and the value of $(-\mathrm{d}\bar{y}/\mathrm{d}s)_{s = 0}$ so obtained is entered in line (N). It should be noted that in the case at hand the value given in line (N) is in units of OD and $(\text{min.}/4)^{-1}$. To convert this latter value into one based upon more general units, *i. e.*, M/min., the value given in line (N) is divided by the factor $b\Delta\tau$, where $b = 98.4$ OD units/M to give the value of v_0 in line (O) which is in units of $M/\text{min.} \times 10^{-4}$. It will be seen that for the experiment being considered $v_0 = 14.20 \times 10^{-4}\ M/\text{min.}$

TABLE IV

VALUES OF (COEFF. $v_0)_{m.n}$ FOR $n = 7$ AND $n = 8$

m	1	2	3	4	5
(Coeff. $v_0)_{m.7}$	2.000	7.000	18.333	47.833	150.633
(Coeff. $v_0)_{m.8}$	1.000	24.000	30.166	72.666	45.933

The fourth part of Table II, *i. e.*, below the third dashed line, is devoted to an estimate of the variability of the value of v_0 obtained immediately above. The values of $L_{m,n}$ in line (P) are those given in Table I. The quantity $\sigma(-d\bar{y}/ds)_{s=0}$ is evaluated as the product of the values given in line (H) and (P) of column $P_3(s)$ and is entered in line (Q). The quantity σ_{v_0} is then evaluated as the quotient of the value given in line (Q) divided by the factor $b\Delta\tau$. The value of σ_{v_0} entered in line (R) is seen to be $\pm 1.04 \times 10^{-4}\ M$/min. which leads to a final value of $v_0 = 14.2 \pm 1.0 \times 10^{-4}$ M/min.

The primary experimental data employed in the example given immediately above are presented in Fig. 1 as is the curve which is a graphical representation of the polynomial selected to represent the experimental data in the above evaluation operation. A line of slope $v_0 = 0.5558$ OD/(min./4) is also presented in Fig. 1 in order to demonstrate the difficulty of producing this latter line by visual inspection.

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[20] R. J. Foster, R. R. Jennings and C. Niemann, THIS JOURNAL, **76**, 3142 (1954).

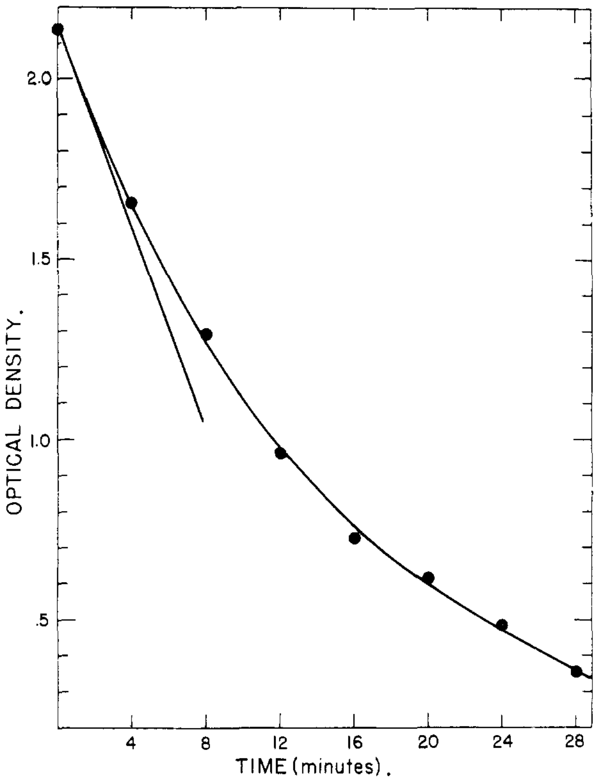

Fig. 1.—Graphical representation of certain of the results given in Table II. Solid circles are the experimental points, curve is the polynomial used for representation of the experimental data, line is the line whose slope is equal to the computed initial velocity.

For an experiment involving nine observations, *i. e.*, for the case where $n = 8$, a form similar to that described by Table II may be prepared. However, it should be noted that for the case of $n = 8$ all of the constant coefficients will have values different than those given in Table II. For the nine point experiment the necessary values of $P_0(s)$, $P_1(s)$, etc., are given in reference 12 and the values of $L_{m,n}$, $(S_m)^{1/2}$ and (coeff. $v_0)_{m,n}$ in Tables I, III and IV. The values of m and of $(n - m)$ will be obvious and the values of $t_{(n-m),\alpha}$ may be obtained from reference 17. The choice of the significance level, *i. e.*, the value of α, is determined largely by experience.

During the past year the polynomial approximation procedure described in this communication has been used to evaluate the initial velocities of several hundred enzyme catalyzed reactions. It has been found to be particularly useful in those cases where sufficient experimental data were not available to evaluate the enzyme-reaction product dissociation constants, where systems were being studied under conditions which appeared to preclude the usual steady state approximations and where the nature of the study, *e.g.*, the preliminary evaluation of the inhibition constants of competitive inhibitors or initial studies of the effect of extrinsic parameters upon certain enzyme catalyzed reactions, did not justify the development of a complete rate equation for the basic reaction system. With respect to future applications of the polynomial approximation procedure, it should be noted that this procedure affords a basis for accepting or rejecting a particular reaction mechanism provided that the primary data are free of systematic errors. If a particular reaction mechanism is assumed and values of the initial velocities are calculated on the basis of the corresponding rate equation then if the postulated rate equation is a valid one, one should observe lower values for σ_y and σ_{v_0} than are observed when the same experimental data are evaluated by the polynomial approximation procedure. If this is not found to be the case, then the postulated reaction mechanism can be rejected.

Finally with regard to the labor involved in performing the computations required by the polynomial approximation procedure, it has been our experience that the evaluation of a limited amount of experimental data with the aid of the block form described above and a desk calculator is no more time consuming than many graphical procedures.[4-9] Furthermore, with larger quantities of experimental data the calculations required by the polynomial approximation procedure can be performed with the aid of a digital computor, such as the IBM 604, with a concomitant decrease in computational time and an increase in computational accuracy.

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Pasadena 4, California