or Norwalk viruses in tissue culture. Preliminary evidence suggests that the gastroenteritis antigen is the etiologic agent of "Norwalk" gastroenteritis and belongs to the class of viruses known as parvoviruses (*9*); studies by Provost *et al.* (*10*) suggest that the hepatitis A virus is also a parvovirus. We could not demonstrate a serologic response to the Norwalk gastroenteritis antigen with paired serums from two patients with hepatitis A, nor could we demonstrate a rise in antibody to hepatitis A antigen with paired serums from two volunteers infected with the gastroenteritis agent (Table 1). Thus, the two antigens do not appear to be serologically related.

In a previous study a virus was recovered from cell cultures inoculated with specimens from the Illinois volunteers (*11*). This virus was subsequently shown to be a parvovirus and to be related to the latent rat virus complex of agents (*12*). Although this isolate was most likely a contaminant unrelated to hepatitis A, the possibility of its association with the hepatitis A antigen was examined with guinea pig hyperimmune serum to the Kilham strain of latent rat virus and particulate antigen of rat virus grown in cell culture (*13*). In IEM studies done under code, a convalescent-phase hepatitis A serum from the Illinois study did not react with rat virus antigen but did with its homologous hepatitis A antigen; and the hyperimmune rat virus serum did not react with the hepatitis A antigen but did with its homologous rat virus antigen. Thus we demonstrated that the hepatitis A antigen was not related to rat virus and, in addition, confirmed the lack of an etiologic relationship of rat virus to hepatitis A.

Cross *et al.* reported particulate fecal antigens thought to be related to hepatitis A infection. However, one of these was smaller (15 to 25 nm) than the hepatitis A antigen (27 nm) whereas the other was larger (40 to 45 nm), and the latter appeared to be serologically related to hepatitis B antigen (*14*). Reagents are not now available to determine whether the hepatitis A antigen described here is antigenically related to the fecal antigens described by Cross.

Commercial immune serum globulin protects against or modifies hepatitis A illness. To determine whether such preparations contained antibody to the hepatitis A antigen, we tested two lots of immune serum globulin, prepared by different manufacturers: one was rated as being strongly positive for such antibody (3 to 4) (Fig. 1, E and F), and the other was found to have a moderate quantity (rated 2) of antibody. Testing for antibody to hepatitis A antigen may prove useful in the standardization of potency of immune serum globulin to be used in the prevention of hepatitis A infection.

Our data suggest that the 27-nm particle visualized in a stool filtrate derived from a patient with hepatitis A may be the etiologic agent of this disease. In addition, the discovery of this viruslike antigen in the stools of patients with hepatitis A, and the development of a serologic technique with which to detect antibody to it, provide, for the first time, a means of diagnosing and studying hepatitis A.

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References and Notes

1. B. S. Blumberg, H. J. Alter, S. Visnich, *J. Am. Med. Assoc.* **191**, 541 (1965); B. S. Blumberg, B. J. S. Gerstley, D. A. Hungerford, W. T. London, A. I. Sutnick, *Ann. Int. Med.* **66**, 924 (1967); A. M. Prince, *Proc. Natl. Acad. Sci. U.S.A.* **60**, 814 (1968).
2. S. Krugman, R. Ward, J. P. Giles, *Am. J. Med.* **32**, 717 (1962); S. Sherlock, *Diseases of the Liver* (Davis, Philadelphia, ed. 4, 1968), pp. 318–319.
3. J. D. Almeida and A. P. Waterson, *Adv. Virus Res.* **15**, 307 (1969).
4. A. Z. Kapikian, R. G. Wyatt, R. Dolin, T. S. Thornhill, A. R. Kalica, R. M. Chanock, *J. Virol.* **10**, 1075 (1972).
5. J. D. Boggs, J. L. Melnick, M. E. Conrad, B. F. Felsher, *J. Am. Med. Assoc.* **214**, 1041 (1970); S. Krugman, J. P. Giles, J. Hammond, *ibid.* **200**, 365 (1967).
6. L. J. Morse, J. A. Bryan, J. P. Hurley, J. F. Murphy, T. F. O'Brien, W. E. C. Wacker, *ibid.* **219**, 706 (1972); the hepatitis outbreak in American Samoa was studied by Dr. L. Rosen (unpublished data).
7. J. L. Lander, J. P. Giles, R. H. Purcell, S. Krugman, *N. Engl. J. Med.* **285**, 303 (1971).
8. R. H. Purcell, J. L. Gerin, P. V. Holland, W. L. Cline, R. M. Chanock, *J. Infect. Dis.* **121**, 222 (1970); J. H. Hoofnagle, R. J. Gerety, L. F. Barker, *Lancet* **1973-II**, 869 (1973).
9. A. Z. Kapikian, J. L. Gerin, R. G. Wyatt, T. S. Thornhill, R. M. Chanock, *Proc. Soc. Exp. Biol. Med.* **142**, 874 (1973); R. Dolin, N. R. Blacklow, H. DuPont, R. F. Buscho, R. G. Wyatt, J. A. Kasel, R. Hornick, R. M. Chanock, *ibid.* **140**, 578 (1972).
10. P. J. Provost, O. L. Ittensohn, V. M. Villarejos, J. A. Arguedas G., M. R. Hilleman, *ibid.* **142**, 1257 (1973).
11. J. L. Melnick, W. Boucher, J. Craske, J. Boggs, *J. Infect. Dis.* **124**, 76 (1971).
12. R. R. Mirkovic, V. Adamova, W. Boucher, J. L. Melnick, *Proc. Soc. Exp. Biol. Med.* **138**, 626 (1971).
13. Kilham strain of latent rat virus cell culture grown (complement fixation titer = 8) and guinea pig antiserum to the rat virus (complement fixation titer = 5260).
14. G. F. Cross, M. Waugh, A. A. Ferris, I. D. Gust, J. Kaldor, *Aust. J. Exp. Biol. Med. Sci.* **49**, 1 (1971).
15. We thank Drs. M. Conrad, J. Boggs, S. Krugman, M. Oxman, and L. Rosen for serum specimens from hepatitis A patients; Dr. M. D. Hoggan for the rat virus reagents; Dr. R. Wyatt for the gastroenteritis reagents; and Dr. L. Barker for the antiserum to the core of the Dane particle; and A. Kalica, J. Valdesuso, L. Kendrick, and D. Bertran for technical assistance.

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Probit Transformation: Improved Method for Defining Synchrony of Cell Cultures

Abstract. *Cell numbers can be converted to probits that are used to compare the degree and timing of synchronized cell cultures. Data from synchronous cultures of* Chlorella pyrenoidosa *have been analyzed by this method by means of a readily available computer program. The method can be used with any biological system that generates normal sigmoidal data.*

The use of synchronous cultures of algae has been of value in numerous investigations of photosynthetic activity, pigment synthesis, nucleic acid synthesis, and changes in metabolism [reviewed in (*1*)]. A problem often encountered with synchronous cultures of microorganisms has been the quantitative comparison of the synchrony of different cultures, because the cell counts, when plotted against time, describe a sigmoidal curve rather than a straight line. In describing synchrony it is not sufficient to describe the growth conditions, moreover, because even when similar procedures are used the synchrony often varies between experiments. To circumvent this problem, some investigators used parameters that were a measure of the time required for completion of cell division (*2*). These numbers are not easily obtained and do not have the required precision. Engelberg (*3*) later introduced a more quantitative method. In the improvement introduced by Spencer *et al.* (*4*), the change in cell number was monitored every ½ hour and the resulting curve analyzed statistically. This method has the advantage of increased precision, but the necessity of making frequent cell counts for each culture at specific time intervals makes it impractical for routine use.

A simpler solution follows from the fact that the change in the rate of cell

division follows a normal distribution function, with the maximum rate of cell division at the midpoint of the curve. The time curve for the total cell number is the integral of the cell division rates up to that time and, therefore, has the same shape as the integral of a normal probability curve. For this reason, the sigmoidal growth curves from various experiments can be easily compared by converting the cell numbers to probits. This transformation converts a normal sigmoidal curve to a straight line (*5*). In the case of cell division, the plot of probit against time produces a straight line having a midpoint corresponding to the peak of the cell division rate (probit of 5.0), with the reciprocal of the slope equaling the standard deviation of the cell release rate. These two parameters, the time of maximum cell division and the spread of the cell release rates around this midpoint, are all that are needed to define degree of synchrony. These parameters can be thought of as analogous to the wavelength of the absorption maximum and the half-bandwidth commonly used in spectroscopy.

Figure 1a shows the sigmoidal curve obtained by plotting percentage of cell division against time for a synchronous culture and a subculture of *Chlorella* measured 2 days apart. The cells in the experiment were synchronized by prior treatment with alternating illumination of 14 hours of light and 10 hours of dark for 7 days. Daily dilutions were used after the third day to keep the initial cell number constant. Cell number per milliliter was determined with a model B Coulter counter (*6*). Figure 1a shows only that all divisions occurred within a single 4-hour span in the life cycle. Figure 1b is a plot of probit values against time (in hours) of the cells described in Fig. 1a. The midpoint, probit equals 5.0, is 9.8 hours after darkening the culture, and the probit slope is 1.75 hr^{-1}, which corresponds to a standard deviation of 0.57 hour for cell release. These two values define the synchrony of the culture. Time zero, the point of reference, was the end of the 14-hour light period. Selection of a different zero time affects only the cell division midpoint. The standard deviation for cell division is a critical parameter for describing the synchrony of the culture. If the cell division spread is too great, physiological changes that reverse themselves every few hours during the cell cycle would be averaged out and not seen. The time required for 95 percent of the cells in a synchronous culture to complete division is a span of ± 2 standard deviations. In the culture described in Fig. 1, 95 percent of the cells divided in a 2.28-hour span of the cell cycle (4×0.57 hour).

The introduction of the probit transformation by Bliss (*7*), a simplification of Gaddum's (*8*) method of using the normal equivalent deviate, provided a useful tool for describing dose-response effects of various chemicals by determining the dose that would affect half of the organisms tested, and also the effect of different doses on the organisms tested (spread of effectiveness). The probit value corresponds to a given probability in a normal distribution curve having a variance of 1.0 and a mean of 5.0. As the probability approaches 0 or 1.0, the probit values decrease or increase without limit. For drug dose curves, the logarithm of the drug concentration is used to produce the normal response curve. With cell division data, we found that the dosage value of time was best left linear rather than logarithmic.

To convert cell number (N_i) to probits, the proportion of objects responding (P_i) is calculated as follows:

$$P_i = (N_i - N_0)/(N_e - N_0) \quad (1)$$

where P is the proportion responding; N_i is the number of cells per milliliter at time i; N_0 is the number of cells per milliliter before division has started; and N_e is the number of cells per milliliter after all division is completed. Since the number of cells counted is large, this proportion can be used as probability. The proportion value is converted to probit directly (*9*) or, alternatively, converted to a normal deviate by means of a normal probability table and the probit obtained by adding 5.0 to the value for the normal deviate. A simple approximation devised by Hastings (*10*) for computer use calculates normal equivalent deviate from the proportion, and the respective probits are calculated from these values. The error in this approximation is less than 1 in 10^7. The formula for the calculation, not valid for probability values of 0 and 1.0, is as follows:

$$k = c[g - (2.515517 + 0.802853g + 0.010328g^2)/(1.0 + 1.432788g + 0.189269g^2 + 0.001308g^3)] \quad (2)$$

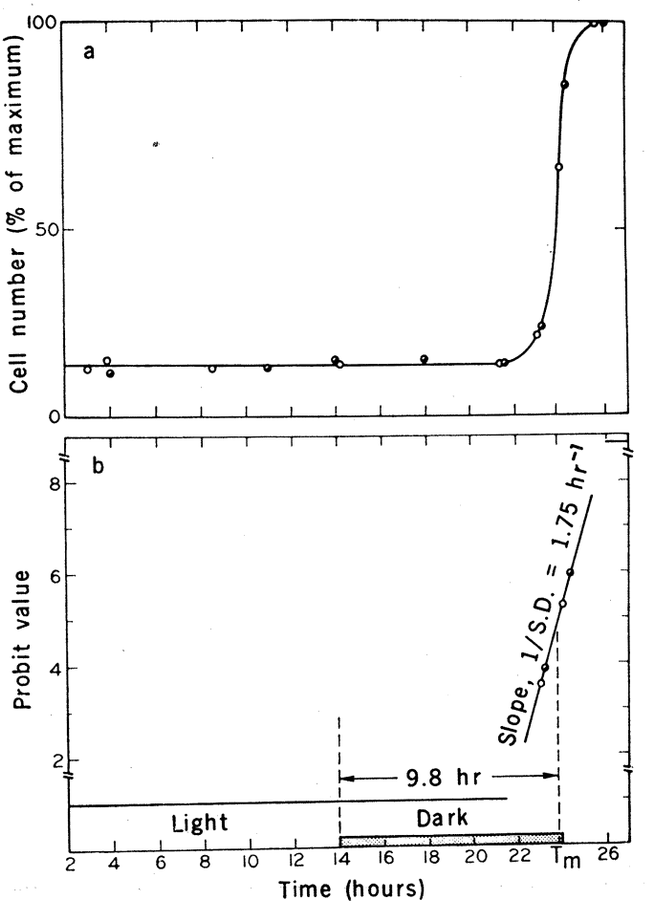

Fig. 1. (a) Cell number of two continuous synchronous subcultures of *Chlorella pyrenoidosa* at various stages of the life cycle. These cultures were exposed to the same synchronization treatment and were measured 2 days apart; ○ and ◑ indicate the different cultures. (b) Probit values of the data in (a) plotted against time in the life cycle; S.D., standard deviation.

where k is the normal equivalent deviate; c is -1 for proportions under .5; c is $+1$ for proportions above .5; and g is $[\ln(1/p_i^2)]^{1/2}$. Here, p_i equals P_i (the proportion responding) for P_i values of .5 or less, and equals $(1.0 - P_i)$ for values greater than .5. The required probit value Y, is

$$Y = k + 5 \quad (3)$$

Finney (*11*) provided a computer program for calculating probit values. This program calculates the y intercept, the probit slope, the chi-square reliability values, and degrees of freedom for each analysis. This maximum likelihood iteration program converges quickly and stops when two successive iterations produce changes less than 10^{-7}. The program is part of the standard International Business Machines scientific subroutine package (*12*) and is included in the library subroutines at most computer centers.

To use this program for analysis of cell division, the cell number data, N_i, is converted to proportion responding, R_i.

$$R_i = (N_i - N_0)/N_0 \quad (4)$$

The total number of cells at the end of the measurement is converted to the total number of subjects tested, U.

$$U = (N_e - N_0)/N_0 \quad (5)$$

These formulas are then added to the beginning of the probit computer program to convert the experimental data into the form needed for the program (*13*); or a separate program can be written with the converted Finney program as a subroutine.

The computer analysis of the synchronized cultures of *Chlorella* is shown in Table 1. All cultures listed were synchronized by light-dark illumination, but the cell densities and light intensities varied between experiments except for the last four subcultures. The y intercept (a) and the slope (b) are part of the printout of the program. The mean division time, T_m, was calculated by the following formula:

$$T_m = (5 - a)/b \quad (6)$$

The chi-square values and the degrees of freedom were used to determine the goodness of fit of the data to the theoretical normal curve. These calculations show the power of the probit method in determining degree of synchrony. The midpoints of the rate of cell division occurred at essentially the same time, but the standard deviation of cell division rates varied because of culturing conditions. If there had been more than one synchronous population of cells in the culture, the plot of probits against time would show two or more linear regressions with time, and the accompanying high chi-square values would have indicated synchrony problems.

Table 1. Analysis of 19 synchronous cultures of *C. pyrenoidosa* by means of the converted Finney program (*11*). The time of maximum cell release was calculated from Eq. 6. The error expressed is the standard error of the mean.

Intercept (a)	Slope (b)	t, mean (hours)	Probability of fit (%)
13 hours light, 11 hours dark			
− 5.21	0.98	10.44	95
− 1.76	0.69	9.84	97
− 7.84	1.20	10.74	> 99
− 6.73	1.13	10.41	94
− 5.35	1.00	10.31	> 99
− 11.45	1.65	9.98	87
− 7.02	1.17	10.25	76
− 9.29	1.39	10.29	*
		10.28 ± 0.10	
14 hours light, 10 hours dark			
− 13.29	1.69	10.81	88
− 8.58	1.28	10.65	97
− 3.03	0.78	10.26	84
− 6.38	1.18	9.63	97
− 10.19	1.66	9.17	96
− 6.96	1.26	9.53	62
− 6.94	1.23	9.71	81
− 7.35	1.23	10.01	26
− 11.78	1.70	9.89	*
− 12.52	1.79	9.81	*
− 10.13	1.55	9.75	*
		9.93 ± 0.13	

* Indicates not enough measurements for chi-square analysis.

The data of Spencer *et al.* (*4*) were also analyzed by the modified Finney program. The standard deviation obtained with the computer program was 0.63 hour as compared to 0.61 hour that they obtained, and the mean time of cell division was the same, 10.8 hours. Theoretically, if the time intervals of their measurement had been short enough, the two methods would have been equivalent. The chi-square analysis gave > 98 percent correlation of their data with the probit transformation. The ease of computation of the probit values and the important advantage that the cells do not have to be counted at specific time intervals make the probit analysis an excellent method for comparing cell synchronies.

Probit analysis can be applied to analyze any biological system that yields normal sigmoidal data. In the case of cell synchrony, any measurable quantity dependent on the cell cycle can be converted to probits and used to determine the synchrony of the system. With microorganisms, cell number is the easiest quantity to measure. Probit analysis of cell numbers produces, probit slope and midpoint, which accurately describe the time of maximum rate of cell division and the spread of cell division. When probit values are used with other parameters such as the number of autospores released per mother cell, temperature, pH, and synchronization techniques, synchronized cultures can be completely defined and easily compared to each other.

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References and Notes

1. H. Tamiya, *Annu. Rev. Plant Physiol.* **17**, 1 (1966); A. Pirson and H. Lorenzen, *ibid.*, p. 439; E. Zeuthen, Ed., *Synchrony in Cell Division and Growth* (Interscience, New York, 1964); A. San Pietro, Ed., *Methods in Enzymology* (Academic Press, New York, 1971), vol. 23, sect. 1, part b, pp. 53–96.
2. O. H. Scherbaum, *J. Protozool.* **6**, 17 (1959); T. W. James, *Ann. N.Y. Acad. Sci.* **90**, 550 (1960).
3. J. Engelberg, *Exp. Cell Res.* **23**, 218 (1961).
4. H. T. Spencer, R. R. Schmidt, C. Y. Kramer, W. E. C. Moore, K. W. King, *ibid.* **25**, 485 (1961).
5. D. J. Finney, *Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve* (Cambridge Univ. Press, London, 1953).
6. B. M. Sweeney and F. W. Hastings, in *Synchrony in Cell Division and Growth*, E. Zeuthen, Ed. (Interscience, New York, 1964), pp. 579–587.
7. C. I. Bliss, *Science* **79**, 38 (1934).
8. J. H. Gaddum, *Spec. Rep. Ser. Med. Res. Counc. Lond. No. 183* (1933).
9. R. A. Fisher and F. Yates, *Statistical Tables for Biological, Agriculture and Medical Research* (Oliver and Boyd, London, 1953), pp. 60–62.
10. C. Hastings, Jr., *Approximations for Digital Computers* (Princeton Univ. Press, Princeton, N.J., 1955), p. 192.
11. D. J. Finney, *Probit Analysis* (Cambridge Univ. Press, London, 1971).
12. *IBM application program GH20-0205-4*, System/360 scientific subroutine package version III (International Business Machines, White Plains, N.Y., 1970), program 360-A-CM-03X, p. 44.
13. The do loops in the program after this calculation must be initialized at 2 and "K" must be set equal to K − 1, or the computer program will terminate due to calculations of probability values of zero and one. The calculation for the number of degrees of freedom at the end of the program also must be decreased by one. Cell numbers equal to the initial cell number, N_0, and final cell number, N_e, cannot be used with this program.
14. R. A. Fisher, *Statistical Methods for Research Workers* (Oliver and Boyd, London, 1936), p. 340.
15. This is Carnegie Institution of Washington–Department of Plant Biology publication No. 495.

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