perch, *Phanerodon furcatus* (Girard), were investigated. The results given in Table 1 show the species specificity of the alarm reaction.

Initial investigations on fractionation of the top smelt alarm substance indicate that the substance can be partially extracted from its water solution with petroleum ether (boiling point, 30° to 60°C) (*8*).

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Semilogarithmic Plots of Data Which Reflect a Continuum of Exponential Processes

Abstract. A set of experimental data which describes a curve on semilogarithmic paper may reflect the operation of a continuum of exponential processes, particularly in biological systems where variability is expected. The "backward projection" technique for graphic separation of exponential processes is most misleading when applied to systems in which the processes are distributed so that the standard deviation of halftimes is large compared to the mean halftime.

If a measured quantity decreases at a rate which is proportional to its value at any time, a single exponential process can be said to be taking place, and the data will describe a straight line when plotted on semilogarithmic paper. When a set of experimental data does not give a straight line, some investigators employ a graphical analysis technique which can be called "backward projection" to separate the semilog curve into two more straight components. The terminal portion of the curve is projected backward until it intersects the *y*-axis, and the ordinate values of the projected line are subtracted from the ordinate values of the original curve. If a plot of the log of the difference versus time is a straight line, the original curve can be said to be the sum of two exponential processes. If the second plot is also curved, the backward projection procedure can be applied again and again until only straight lines remain. In study of the elimination of a substance from an animal's tissues, the derived straight lines are usually assumed to be due to independent compartments which eliminate the substance with different rate constants. The slope of a particular line is taken as characteristic of the rate constant of its compartment, and the *y* intercept as an index of the relative size of the compartment. Usually no more than three components are found by backward projection, and the values for slopes and intercepts cannot be considered exact because of inaccuracies inherent in the fitting of lines by eye.

It may often be more acceptable to envision a great many components with rate constants which are distributed continuously, instead of the two or three discrete components obtained by the backward projection technique. For example, backward projection on nitrogen wash-out curves suggests two compartments in the lungs of a normal subject, one well ventilated and one poorly ventilated. This is physiologically unreasonable; the functions of various parts of the lungs probably occur in a continuum with some parts very well ventilated, some very poorly ventilated, but most probably clustered around an average.

Figure 1 shows a curve on semilog paper (heavy curve) which was fabricated by backward projection in reverse. The curve is the sum of the ordinate values of the light lines which represent eight components with consecutive integers for halftimes, as shown in the histogram inset. The histogram approximates a continuous normal distribution. The two dotted lines in Fig. 1 are components which were derived by backward projection on the composite curve. The curve is compatible with either the eight "true" components or the two "derived" components.

Figure 2 is an illustration of a semilog curve fabricated from an asymmetric distribution such as may occur quite widely in nature. For example, most of the subunits in the lung of a patient with pulmonary disease may ventilate with a relatively small halftime, but there may be a tail of poorly ventilating units which decrease in number as halftime increases.

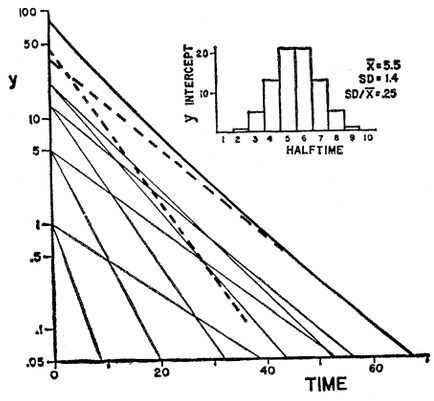

Fig. 1. Fabrication of a curve for a multicomponent system (an approximation of a continuously distributed system). The heavy curve is the sum of the ordinate values of the eight light lines which have *y* intercepts and halftimes shown in the histogram. When the backward projection technique was applied, the curve appeared to be the sum of the two dotted lines.

Semilog curves were fabricated from many different distributions with up to 20 components. When the backward projection technique was applied with ordinary care and accuracy, all the multicomponent systems appeared to be systems with two or three components. The only exceptions were asymmetric distributions with extremely high variance of halftimes; in these, four components were separable. The *y* intercepts and halftimes of the derived com-

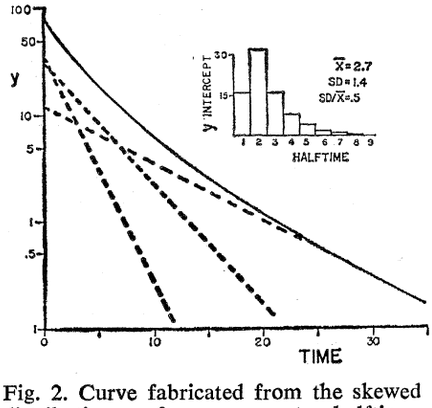

Fig. 2. Curve fabricated from the skewed distribution of component halftimes shown in the histogram. Three components (dotted lines) were isolated from the composite curve by the backward projection technique.

ponents depended on the number of logarithmic cycles through which the curve was carried. Thus when the curve of Fig. 1 was fabricated on beyond three cycles, the following components were derived by backward projection: (y intercept : halftime)—four cycles, 40 : 7 and 40 : 4; five cycles, 28 : 7 and 52 : 4; and six cycles, 22 : 8 and 58 : 5.

Sometimes two separate processes may be expected a priori, as when a tracer molecule is eliminated from both intracellular and extracellular fluids, but contrary to expectation, the actual system may be a bimodal distribution of components because of variance within the two apparently separate categories. For example, the rates of elimination of the tracer molecule from some subunits which would be classed as extracellular fluid on anatomic or physiologic grounds may overlap rates of elimination from some subunits of the intracellular fluid.

If it is assumed that the experimental material being studied will exhibit variance to some degree (as is almost always the case in biology), a curve on a semilog plot may result from operation of one of four types of systems: (i) a single-mode distribution of exponential processes, of which Figs. 1 and 2 are examples; (ii) a multi-mode distribution with so little separation between modes that components derived by backward projection are not identified with the modes; (iii) a multi-mode distribution with enough separation of function that backward projection correctly separates the modes—that is, the modes operate as isolated components; (iv) a complex of process types—for example, kidney excretion of certain substances may sometimes be due to exponential processes acting simultaneously with linear, "active transport" processes.

The concavity of a semilog curve from a single-mode distribution of exponential processes is determined by the relative magnitude of the standard deviation (SD) and the mean of the distribution; the larger the SD/mean ratio, the greater the curvature (for example, the curve in Fig. 2 is more concave than the curve in Fig. 1). Conversely, the smaller the SD/mean ratio, the more nearly the composite curve resembles a single exponential process; if the histogram of Fig. 1 is moved to the right to increase the mean value without altering the SD, the resulting curve on semilog paper becomes straighter and straighter.

When backward projection is applied to the almost-straight curves from distributions with small SD/mean ratios, they tend to separate so that the largest derived component has a halftime approximating the mean halftime of the distribution, but curves which are concave tend to give two or three components of similar magnitude unless the data are carried through many log cycles. Therefore, the results of backward projection will be most misleading if the exponential processes responsible for the curve are distributed with a large SD/mean ratio; the derived components suggest two or three isolated processes of similar magnitude, where in fact there is only a single type of process, but the subunits of the system vary in their rate of operation.

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Chemiluminescence of Firefly Luciferin without Enzyme

Abstract. We have been able to produce chemiluminescence in firefly luciferin without utilizing the enzyme luciferase. Following the analogous mechanism of the chemiluminescence of luminol in the organic solvent dimethyl sulfoxide, we have prepared synthetically the methyl ester of luciferin, the phosphate ester of luciferin and luciferyl adenylate by condensation in dimethyl sulfoxide with Khorana's reagent, dicyclohexylcarbodiimide and diazomethane, phosphoric acid, and adenylic acid respectively. These compounds in dimethyl sulfoxide in the presence of base emit a bright chemiluminescence. Like in vitro enzymatic bioluminescence, the luciferyl adenylate chemiluminescence emission spectrum is dependent upon *p*H.

The biochemical steps leading to the enzyme-catalyzed emission of light by firefly luciferin have already been described (*1*). Adenosine triphosphate (ATP) is required to form the "active luciferin-enzyme complex" ($E \cdot LH_2$-AMP), which can then react with molecular oxygen resulting in the emission of a yellow-green band with a peak at 562 mμ. Rhodes and McElroy (*2*) have also shown that synthetically produced luciferyl adenylate (LH_2-AMP) reacts with the enzyme luciferase to produce light in the absence of ATP. We have now been able to demonstrate the nonenzymatic chemiluminescence of LH_2-AMP as well as

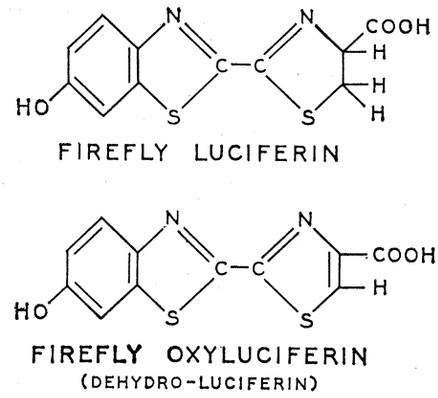

Fig. 1. Chemical structures of firefly luciferin and dehydroluciferin.

that of the phosphate and methyl esters of luciferin (*3*).

The structure of firefly luciferin (*4*) is shown in Fig. 1. We are reasonably certain on the basis of synthesis of other analogs of luciferin (LH_2) that LH_2-AMP is formed by the condensation of adenylic acid (AMP) at the carboxy group. By analogy with the chemiluminescent oxidation of luminol (*5*) the abstraction of one of the hydrogen atoms in the 1- or 2-position in basic solution could then permit oxygen attack which results in an excited state of the product molecule. Since LH_2-AMP is extremely labile in aqueous alkaline solution we have worked primarily in the strongly hydrogen-bonding organic solvent, dimethyl sulfoxide. This solvent is also extremely efficient for the chemiluminescent reaction of luminol.

Firefly luciferin was condensed with adenylic acid, metaphosphoric acid or diazomethane in dry pyridine with dicyclohexylcarbodiimide, according to the method of Khorana (*6*). We have since found that the yields are considerably higher if the reaction is performed in dimethyl sulfoxide. An aliquot was delivered to 2 ml of dimethyl sulfoxide in a 10- by 75-mm test tube mounted in front of a 1P21 phototube. Chemiluminescence was obtained upon addition of a solid pellet

Table 1. Relative peak chemiluminescence intensity.

Compound	Intensity
Luciferyl adenylate (LH_2-AMP)	100
Luciferyl inosinate (LH_2-IMP)	4
Luciferin (LH_2)	1
Dehydroluciferyl adenylate (L-AMP)	3
Dehydroluciferin (L)	1
Dimethyl sulfoxide alone	1
Adenylic acid (AMP)	1
Inosinic acid (IMP)	1