which appear in Table 1, show that the animals in group 1 made significantly more approaches to the ball than those in group 2 ($p = .02$). In both groups, however, the number of approach responses to the ball was significantly greater ($p = .01$) than chance; this indicates that imprinting occurred in both groups. Where a chick chose the ball on both trials of the test, it uttered contentment tones, hovered under the ball, and made distress calls in an appropriate manner. Responses to the blue ball of group 2 chicks that chose the ball on both trials of the test could not be distinguished from responses of chicks exposed to the normal imprinting procedure. Animals in group 3 exhibited no preference for either stimulus at a significant ($p = .01$) level.

It is concluded, therefore, that imprinting does occur when following is involuntary. In each case where a chick was pulled by the ball it demonstrated great distress by giving the appropriate call and struggling violently to resist being pulled. Hess's (*1*) "law of effort" states that imprinting strength is a function of the amount of energy expended in making the following response. In this instance imprinting also occurred when an extreme effort was made to resist following—that is, to move in the direction opposite to that of the imprinting object (*3*).

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9 October 1962

Alarm Reaction of the Top Smelt, Atherinops affinis (Ayres)

Abstract. The "alarm" substance of the top smelt, *Atherinops affinis* (Ayres), has been isolated by extraction with methanol or ether from suffocated top smelt. These concentrated extracts, when introduced into an aquarium containing top smelt, induce a strong alarm reaction in the fish, characterized by rapid swimming, jumping, and often severe seizures. The fact that extracts from white surfperch, *Phanerodon furcatus* (Girard); surf smelt, *Hypomesus pretiosus* (Girard); northern anchovy, *Engraulis mordox* (Girard); or Pacific herring, *Clupea pallasi* (Valenciennes) caused only mild excitation in the top smelt indicates the species-specificity of the alarm reaction.

The exceptional sensitivity of the olfactory senses of marine animals is well established (*1*). Several workers have shown that the ability of salmon to return to their home stream is, at least in part, an olfactory response to differences in organic substances in various streams (*2*). Others have demonstrated the salmon-repellent action of a substance found in human and sea-lion skin when present at a concentration of 1 part in 8×10^{10} parts of water (*3*). This substance was later thought to be the simple amino acid L-serine (*4*).

As a means of warning other members of a school, alarmed fish communicate fright by releasing a chemical substance into the water. This alarm reaction was investigated by von Frisch (*5*) with minnows. Extracts of minnow skins induced a fright reaction in the fish; the minnows hovered at the bottom of the aquarium and refused food. Hüttel (*6*), in investigating the chemical nature of this material, concluded that the "fright" substance is a purine or pterine-like compound.

A recent report by Tester (*7*) that hungry sharks are more strongly stimulated by a scent released in the water from alarmed prey fish than from undisturbed prey fish has intensified our interest in investigating the alarm substances from saltwater species of fish.

We have found that the top smelt, *Atherinops affinis* (Ayres), exhibits a strong alarm reaction when dilute water solutions of extracts of that species are added to the saltwater aquarium where these fish are living. The alarm reaction consists of rapid swimming back and forth in the aquarium, darting to the surface, occasionally breaking the surface of the water to the extent of flipping out of the aquarium, and finally, making a rapid swimming motion with the mouth against the aquarium glass. The reaction lasts approximately 3 to 5 minutes. Often top smelt will lapse into a seizure, will quiver, and will sink upside down to the bottom of the aquarium. Although they usually recover after 20 to 30 minutes, occasionally these seizures end in death, which may be caused by the fish's striking the sides of the aquarium.

Methanol extracts of the skins or of the whole top smelt (after the fish had been freshly killed by suffocation) were made, the methanol was removed *in vacuo*, and the semisolid product was dissolved in distilled water for evaluation. After the top smelt had been removed from the methanol they were placed in ether overnight at room temperature, then more of the alarm substance was extracted.

Control experiments with small amounts of methanol and Celite suspended in distilled water to simulate the slight cloudiness of the extracts always yielded negative results. The fish seemed to be unaware of several milliliters of methanol introduced into the aquarium.

There was no response to extracts from top smelt that had been kept chilled for 4 days in ice, or to extracts that had initially evoked a response after these had been stored in distilled water at 0°C for a week. It is suspected that there was bacteriological degradation of the alarm substance. Hüttel (*6*) mentioned such a problem with extracts from minnows.

After the original strong alarm reaction had been induced and the top smelt had resumed their normal swimming behavior (after 3 to 5 minutes), addition of more of the alarm extract had no observable effect on them. However, the next day a strong alarm reaction could again be evoked by adding the alarm extract.

In order to ascertain the species specificity of the alarm reaction to the top smelt extract, the effects of extracts of other species of saltwater fish on the top smelt and on white surf-

Table 1. Data showing species specificity of alarm substances from saltwater fish. Reactions: (−) feeding; (0) no reaction; (+) mild excitation (quick movements, simulated swimming action with mouth against glass); (++) moderate excitation (rapid swimming or shelter seeking); (+++) strong excitation (rapid swimming accompanied by jumping out of the water).

Extract	Dry weight of extracted material (g)	Reaction	
		Top smelt	White surfperch
Extracting agent: methanol			
Top smelt	1.18	++ to +++	0
White surfperch	2.70	0	−
Surf smelt	0.94	0	−
Northern anchovy	2.30	+	−
Pacific herring	1.60	+	−
Extracting agent: ether			
Top smelt	0.49	++ to +++	−
White surfperch	0.80	+	−
Surf smelt	6.43	+	−
Northern anchovy	9.70	0	−
Pacific herring	4.30	+	

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.

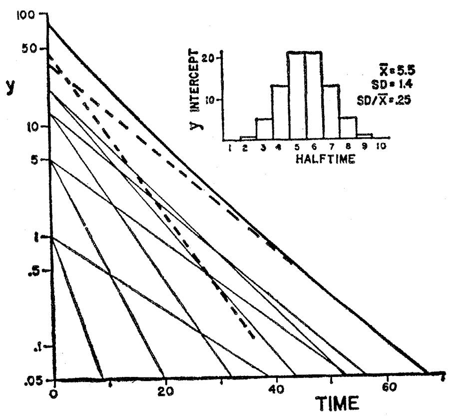

Fig. 1. Fabrication of a curve for a multicomponent system (an approximation of a continuously distributed system). The heavy curve is the sum of the ordinate values of the eight light lines which have *y* intercepts and halftimes shown in the histogram. When the backward projection technique was applied,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

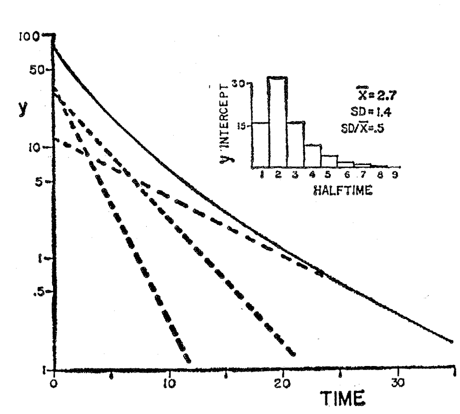

Fig. 2. Curve fabricated from the skewed distribution of component halftimes shown in the histogram. Three components (dotted lines) were isolated from the composite curve by the backward projection technique.