a reinforcement value of each value of P. These requirements were met by first establishing a range of P_D through the use of different sucrose concentrations and a range of P_R through the use of different force requirements on an activity wheel, and then conditioning the animals to press a bar for sucrose concentrations and wheel conditions.

Four female albino rats of the Sprague-Dawley strain, about 200 days old, were used. The apparatus was a modified activity wheel equipped with a brake, a retractable drinkometer, and two bars. In operant drinking sessions, only the tube was free; in operant running sessions, only the wheel was free. During conditioning the appropriate bar was inserted and its corresponding item, tube or wheel, was made contingent upon the bar press. Sessions lasted 10 minutes; all running sessions, operant and conditioning, occurred in the morning, all drinking sessions in the afternoon. Animals were given operant running sessions with a given force requirement and operant drinking sessions with a given sucrose concentration until both behaviors were relatively stable. They then pressed a bar, in the morning for the wheel, and in the afternoon for the tube, with the same stimulus values as in the preceding operant series. Sucrose solutions, in the order of presentation, were 32, 16, and 64 percent by weight; force requirements, in the order of presentation, were about 18 and 80 inch grams. The same conditioning parameters were used in both cases: each time the rat pressed the bar three times, either the wheel or the tube was made available for 15 seconds.

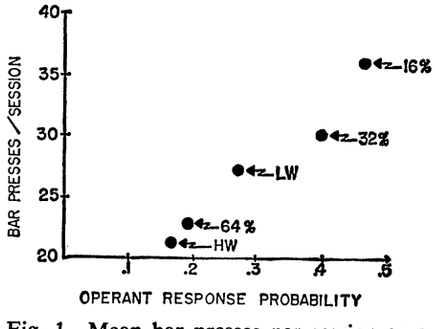

Fig. 1. Mean bar presses per session as a function of the associated operant response probability. The abscissa shows proportion of the operant session for which the animal responded—for example, duration in seconds for which it ran or drank divided by duration of the session. Points are labeled according to the sucrose concentration (16, 32, and 64 percent) or force requirement (light, LW; heavy, HW) that was used to control the operant response probability.

A photoelectric cell, activated each time the wheel turned 90 degrees, measured running; a drinkometer counted licks. Both devices could be used to measure duration of running and drinking by arranging that a precision timer operate continuously when receiving seven or more pulses per second from the drinkometer or four or more pulses per second from the wheel. The procedure eliminates spurious measures due to wheel-rocking and incidental contacts with the drinkometer, since these occur below the prescribed rates.

Figure 1 shows bar presses per session, plotted as a function of the proportion of the session the animals drank and ran, respectively, expressed as the probability of free running and drinking. Thus, a "P_R of .33" indicates that the animal ran or drank for 200 seconds in a 600-second session. Points are averages for the group taken from the last four sessions given at each value of sucrose concentration and force requirement. The order of the points for all animals was the same as that shown in Fig. 1.

The points in Fig. 1 are labeled according to the associated sucrose concentration or force requirement; their rank order on the abscissa shows that the present combination of sugars and force requirements did produce at least partly overlapping probabilities of free running and drinking. Thus the lowest probability of free drinking, produced by the 64 percent solution, fell between the two probabilities of free running. Conversely, the higher probability of running, produced by the light force requirement, fell between the two probabilities of drinking that were associated with the 64 and 32 percent solutions, respectively.

The functional relation shown in Fig. 1 supports the present prediction: bar pressing increased monotonically with the associated operant response probability and did so whether this was a probability of drinking or of running. Not only is bar pressing reinforced by an activity proportional to the operant duration of that activity but, more important, the proportionality is the same for different activities. Figure 1 also suggests that the relation may be linear, although no attempt was made here to determine the function.

An earlier test of the model, using the same behaviors, showed that running reinforced drinking as effectively as, more conventionally, drinking reinforced running. More important, the effective direction of reinforcement was determined by which behavior was relatively more probable in the given interval of time (*5*). The new results thus substantially strengthen the suggestion that reinforcement value is predictable from the proportion of time the animal spends responding, and is so commensurately for different behaviors (*6*).

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4 January 1963

Apparent Concentration Quenching of Morphine Fluorescence

Abstract. *Fundamental fluorescence equations were examined. Deviations from the theoretical were observed by varying the light path length to determine effects on "apparent" fluorescence. It was found that the decrease in emission of morphine solutions with the increase in concentration was caused by absorption effects that prevented excitation of the whole system.*

The formula

$$F = I_0 (1 - 10^{-\varepsilon c b}) \phi \qquad (1)$$

expresses the fluorescence intensity F in solution, where I_0 is the intensity of exciting light, c the concentration, ε the molar absorptivity, b the sample path length, and ϕ the quantum efficiency. All of these values are expressed in appropriate units (*1*). When a fixed-slit instrument is used, for example, the Aminco-Bowman spectrophotofluorometer, the F values are only apparent fluorescence (F'), since factors of detector response and slight differences of optics vary for each instrument (*2*). Corrections for both excitation and fluorescence spectra can be made (*1*, *3*), but the response for each instrument for a constant I_0 should be repro-

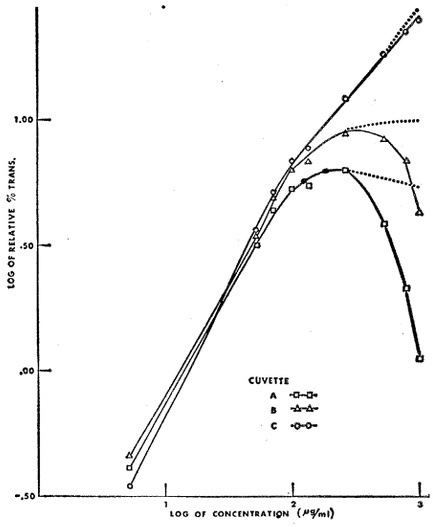

Fig. 1. The log of relative percent transmission (F') versus the log of concentration for various sizes of cuvettes: "A," 46 mm by 10.5 mm square; "B," 40 mm by 7 mm round; "C," 20 mm by 3 mm square; at peak excitation of 285 mμ and peak emission of 350mμ in 0.1N sulfuric acid for morphine.

ducible at a particular wavelength over some range of concentration for below-concentration quenching dilutions.

For the same compound at a particular concentration, F' is proportional to the "effective" optical light path, and as b increases the value of $(1 - 10^{-b})$ approaches unity (Eq. 2). The "effective" optical light path can, in theory, be lengthened by increasing the cuvette width.

$$F' = K\ (1 - 10^{-b}) \qquad (2)$$

where K is the factor for the multiple values, ε, ϕ, I_0, and c.

With the use of 0.1N sulfuric acid solutions of morphine, the 285 mμ peak was used as the major activation to excite maximum emission at 350 mμ (*4*). A second excitation peak observed at 245 mμ may be the actual major one, but the rapid falloff of I_0 below 280 mμ for the xenon lamp would mask this. The plot of the log of F' versus the log of c was previously shown (*4*) to be linear from 0.1 to 100 μg/ml with solutions in the "A" cuvette (described below). At higher concentrations there was a marked decrease in fluorescence which may be interpreted as being concentration quenching of the ionic dimer type theorized by Forster (*5*).

For true quenching it would be expected that a plot of concentration versus fluorescence would be linear for dilute solutions, reaching a maximum with increasing concentrations, and finally decreasing with further change in concentration. The measurement of fluorescence emission at angles less than 90° to the exciting source results in a similar plot, but the range of linearity is extended by several magnitudes (*6*). Since most available spectrofluorometers and fluorometers are used to measure emission at either 90° to the exciting radiation or directly through the solutions, a special instrument or modification (*6*) of existing instruments would have to be used to show that reabsorption at higher concentrations is responsible for "apparent" concentration quenching. In place of modifications, the light path was increased simply by using wider cuvettes.

Various cuvette sizes (*7*) are available with adaptors for the Aminco-Bowman instrument. It was expected that at concentration quenching ranges the fluorescence would decrease in the order of the decrease of cuvette width: "A," 46 mm by 10.5 mm square; "B," 40 mm by 7 mm round; "C," 20 mm by 3 mm square. This would obey Eq. 2. The lens effect for the round cuvette is probably slight for the narrow slits used in these studies. The results shown graphically in Fig. 1 indicate that with the smallest cuvette "C," the F' is greater at high concentrations. Therefore, an absorption effect, and not concentration quenching, accounts for the decreased F' for longer b. Linearity is noted for the "C" cuvette at 100 μg/ml until close to 1000 μg/ml. This is an extension of the utilizable fluorescence range. At lower concentrations, the emission from the "C" cuvette begins to correspond to Eq. 2, and at 5 μg/ml, the emission yield is below that of either of the two others used, thus showing the validity of Eq. 2 for this range.

Excitation peak shifts to higher wavelengths are observed as concentration increases. These shifts of intensity are shown as dotted lines in Fig. 1 and are caused by the same inner filter effect that yields "apparent" concentration quenching. A compound with an absorbance of 0.02 would introduce an inner filter error of about 4 percent (*1*). As the absorption increases, this error increases, owing to the failure of excitation of a large portion of the molecules present. This same phenomenon is dramatically noted in the visual observation of fluorescein solutions at high concentration (10^{-4} to $10^{-5}M$). In obtaining absolute quantum efficiencies (*8*), this effect is avoided by surface emission detection.

The possibility for the oxidation of morphine to pseudo-morphine at room temperature (25°C) in acid is eliminated in these measurements by preparing solutions directly before measurement (*9*; *10*).

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Strontium-90 in Hair

Abstract. *The hair of rats injected with strontium-90 retains a significant amount of the radionuclide. Although the strontium-90 content of hair is variable in these rats and appears to be subject to a variety of influences, determination of the radionuclide content of hair may offer a nondestructive method of estimating strontium-90 in bone.*

Since hair contains strontium as well as a variety of other elements (*1*), we decided to determine whether strontium-90 accumulates in this tissue to an appreciable degree. Accordingly, we assayed the hair of rats with varying body burdens of Sr^{90} and found that their hair contained significant amounts of this radionuclide.

Hair was clipped from groups of 4 to 12 adult rats whose body burden of Sr^{90} had been established by injection on the 300th day of life, or *in utero*. In the latter cases the mothers were injected on the 17th or 20th day of pregnancy.