

caused by respiration;  $y$  is the output of the pacemaker and has a periodic solution. The maxima of  $y$  are considered to correspond to the times of firing of the pacemaker.

Comparison of the predicted and actual heart rate changes has been carried out successfully, showing a good correspondence for a wide variety of breathing modes.

Figure 1 shows a portion of a half-hour simulation record showing heart rate, simulated heart rate, and respiration for various modes of breathing. The simulated heart rate was computed by the analog computer and simultaneously recorded with the actual heart rate (5). The only signal entering the computer from the subject was the subject's respiration in the form of an electric signal proportional to thorax circumference. The actual and simulated heart rate are recorded above each portion of the respiration record. The record was taken with the subject in a resting state, supine; the subject was a 26-year-old male. Each ordinate represents the time between two consecutive heart beats, measuring from  $R$  to  $R$  peak of the  $QRS$  complex of the electrocardiogram. Thus a long line represents a slow heart rate, a short line a fast heart rate. The basic inspiratory and expiratory heart rate transients are shown in the lower parts of the figure.

Parameters of the differential equations represented by the computer remained unaltered during the course of the simulation. Note the correspondence between actual and simulated heart rates. Since the measure taken of respiration is the external circumference of the thorax, some amplitude dissimilarities between the stretch experienced by the organic receptor and our recording device is to be expected. Some nonlinearities in this regard are likely.

Note that a slight dissimilarity is actually theoretically required, since each cardiac cycle in the simulated circuit does not begin synchronously with the actual heart. Consequently, a deep inspiration, for example, does not necessarily begin at the same point in the cardiac cycle in the simulated circuit as in the real heart, thus causing a minor deviation in the transient. Some steady state changes in heart rate with extent of chest expansion are also sometimes found in addition to the transient changes described.

It may be concluded that in the unanesthetized human being, respiratory sinus arrhythmia (the heart rate changes caused by respiration) is initiated by stretch receptors located within the chest and is not primarily caused by hemodynamic factors or central influences. The paradoxical findings regarding the

phasic relation between heart rate and respiration are resolved by showing that the heart rate waves observed are in fact superpositions of biphasic transients of definite shape and duration. As a result of the mathematical analysis, in terms of nonlinear differential equations, it is possible to predict the heart rate changes (both regular and irregular) due to respiration for any manner of breathing in the given subject.

MANFRED CLYNES

Rockland State Hospital,  
Orangeburg, New York

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22 June 1959

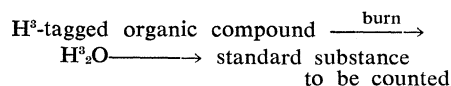
#### Solid-Counting of Octadecane-1- $H^3$

**Abstract.** A solid sample of octadecane-1- $H^3$  was found to show a fairly good proportionality of the counting rate to the amount of the sample. Taking account of the synthetic process, nonvolatility, and spreadability of octadecane, this compound offers a possibility of being used as a standard sample for counting the radioactivity of tritium.

Many investigators have attempted to count the radioactivity of tritium, which emits extremely soft  $\beta$ -rays, by gas-phase (1) and liquid scintillation (2) counting. However, these methods require some special and expensive equipment and complicated procedures. Moreover, it seems inevitable that these methods should suffer from some difficulties—for example, memory effect for the former method and limited applicability for the latter—in performing the measurement. To avoid the memory effect, attempts were successfully made to perform the solid-counting of vegetable oil (3) and  $p$ -aminosalicylic acid (4), both tagged with tritium. A weakness of the use of these two substances, however, arises from limited applicability: The first must be synthesized by adding tritium as a gas to unsaturated oil; the second, by a gas-exposure method in which carrier-free tritium gas is used (5).

Because of these difficulties, it seems desirable to change a sample of organic

compound tagged with tritium to a standard form through a common process such as



To practice good solid-counting, the final product should be (i) prepared easily and quantitatively from  $H^3_2O$ , (ii) nonvolatile at room temperature, and (iii) uniformly spreadable on a suitable surface. Octadecane, synthesized by the reaction of tritiated water and excess octadecyl magnesium bromide, satisfies the first two conditions, and, when it contains a small amount of amphipathic compound, it may also satisfy the third. Thus, this compound seems to be suitable for a standard sample to be counted.

In the present experiment, octadecane-1- $H^3$ , synthesized by the above reaction and containing a small amount of octadecanol, was used. A portion of this substance was spread from benzene solution onto the bottom of a stainless steel pan (24.5 mm in diameter), and the radioactivity was determined by using a  $2\pi$  flow counter (Aloka, type DC-1001). Commercial propane was used as a flow gas (6).

Figure 1 shows a dependence of the counting rate upon the sample thickness. The calculation assumes uniform spreading of octadecane over the bottom of the pan, though this is not always valid. In spite of this rough assumption, the counting rate increased proportionally with the increase of the sample thickness, when the amount of the sample was less than that corresponding to the maximum range of  $\beta$ -rays. Within this region, the counting rate was reproducible for a given amount of the sample, irrespective of distribution of the sample over the bottom of the pan. Beyond this region,

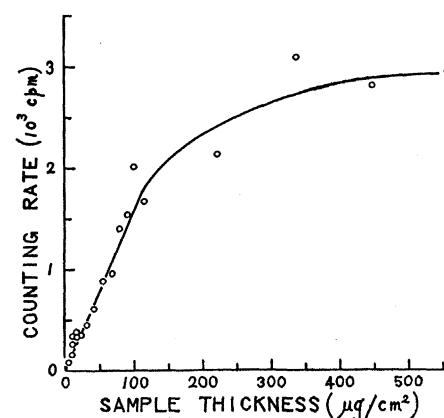


Fig. 1. Dependence of counting rate upon the thickness of sample of octadecane-1- $H^3$ .

however, the counting rate was affected by several conditions, such as an uneven distribution and the surface roughness of samples.

Through all experiments, no memory effect was observed. A memory effect would hardly be anticipated from the low volatility of octadecane. The present results suggest an applicability of the solid-counting method to soft  $\beta$ -rays of tritium-tagged substances, if the sample thickness is chosen to be in a proportional region, as shown in Fig. 1.

MITSUO MURAMATSU  
TSUNETAKA SASAKI

Department of Chemistry,  
Faculty of Science,  
Tokyo Metropolitan University, Japan

#### References and Notes

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10 July 1959

## Electron Transfer and Absorption Spectra of Complexes

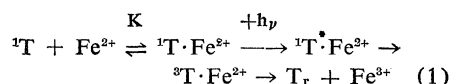
**Abstract.** Occurrence of light saturation in the reaction of thionine (T) with  $\text{Fe}^{2+}$  suggests the existence of  $\text{T}\cdot\text{Fe}^{2+}$  complexes practically indistinguishable by their absorption spectra from free thionine; photoreduction seems to occur by electron transfer in such complexes, delayed by more than  $10^{-5}$  second after light absorption. Both phenomena must be of general significance.

It has been customary to use absorption spectra to decide whether two reagents in solution form a complex or not. It was assumed that the absorption spectrum of the complex must be different from that of its components, at least to the extent to which the spectrum of a dissolved molecule usually is affected by the solvent.

However, Watson (1) had to postulate the occurrence of phenylhydrazine complexes of chlorophyll to account for the effect of this compound on the fluorescence of chlorophyll; and yet no change in the absorption spectrum of chlorophyll could be noted upon the addition of small amounts of phenylhydrazine. Bannister (2) had to make a similar assumption in the case of chlorophyll and ascorbic acid to explain

the kinetics of the photoreduction of chlorophyll by this compound. Weber (3) found that enzymatic removal of the nicotinamide nucleus from diphosphopyridine nucleotide does not affect the absorption spectrum; and the absorption spectrum of ethyl chlorophyllide was found, by Holt and Jacobs (4), to be practically identical with that of chlorophyll (phytyl chlorophyllide); so it was shown that even chemical bonding sometimes leaves the absorption spectrum practically unchanged.

One of us (S.A.) arrived at a similar conclusion in a systematic study of the kinetics of photoreduction of thionine (T) by ferrous ions. The phenomenon of light saturation, unusual in simple photochemical reactions in vitro, which he observed in concentrated ferrous solutions ( $10^{-3}$  mole/liter or more) was most easily explained by postulating, as the main or only photosensitive species, a  $\text{T}\cdot\text{Fe}^{2+}$  complex with a finite velocity of formation in the dark (see reaction 1, where T stands for reduced thionine):



Light saturation is reached, in system I, when the rate of complex formation limits the rate of the photochemical reaction.

Yet, measurements of the absorption spectrum of thionine solutions in the presence of ferrous ions show no evidence of complexing. In fact, Hardwick (5), in a recent study of the same reaction, used this fact to exclude a complex mechanism and to treat the reactions as due to encounters of metastable thionine molecules with free ferrous ions. Schlag (6) also disregarded complex formation in his study of the thionine-iron reaction. Havemann and his co-workers (7), on the other hand, did inquire into the possible existence of complexes, confirmed their existence by measurements of the effect of  $\text{Fe}^{2+}$  ions on fluorescence, and assumed complexes to be the photosensitive species in the interpretation of kinetic data. They derived a value of  $K = 30$  for the complex formation constant.

The kinetic data obtained in our laboratory, and presented in detail in a paper by Ainsworth (8), led to a somewhat higher complexing constant,  $K = 200$ .

In the present report we would like to point out the apparently widespread existence of dye complexes more or less

completely indistinguishable from free dye molecules by their absorption spectra. In the case of the system  $\text{T} + \text{Fe}^{2+}$  (as well as in that of the system chlorophyll + ascorbate), this behavior suggests that electron transfer can occur, in an excited complex, not as an immediate result of light absorption—in which case a new "electron-transfer" band could be expected to appear in the absorption spectrum (9)—but as a delayed effect. As suggested in reaction 1, the dye molecule in the excited complex may be first transferred into a metastable triplet state; this transfer is so effectively catalyzed by the paramagnetic  $\text{Fe}^{2+}$  ions as to quench fluorescence completely. An electron is transferred to the triplet dye from the associated  $\text{Fe}^{2+}$  ion after an average life-time of at least  $10^{-5}$  second; this is indicated by the observation that the reaction is quenched by ferric ions [as well as by thionine molecules (see 10)] in concentrations as low as  $10^{-5}$  mole/liter. (Even resonance energy migration could not account for significant quenching of a singlet excited state by a quencher present in such low concentrations.) Another consequence of this mechanism is the probability of a primary back reaction within the complex; this may explain why the highest quantum yield of the  $\text{T} + \text{Fe}^{2+}$  reaction that we were able to obtain was about 0.3.

The two conclusions—that spectroscopically unrecognizable complexes exist, and that delayed electron transfer occurs in such complexes after electronic excitation of one of the components—appear to be of some general interest.

STANLEY AINSWORTH\*  
EUGENE RABINOWITCH

Photosynthesis Laboratory,  
Department of Botany,  
University of Illinois, Urbana

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\* Present address: Chemistry Department, University of Sheffield, England.

10 August 1959