as cases of classical hemophilia was the prolonged recalcification time corrected to normal in the above system.

It would thus appear that the diagnosis of classical hemophilia can be made with this artificial reagent; diagnosis by this means would have the advantage of simplicity and availability of material (*8*).

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4. I wish to thank Dr. J. P. Soulier and members of his staff for making available the facilities of his laboratory and the plasmas of the various patients studied.
5. The commercial product used was Komplexon III, obtained from A. G. Siegfried, Zofingen, Switzerland.
6. Asolectin is a preparation of mixed soybean phosphatides, obtained from Associated Concentrates, Woodside, Long Island, N.Y.
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15 September 1958

## Fusion Contour for Intermittent Photic Stimuli of Alternating Duration

*Abstract.* It is generally thought that fusion of intermittent photic stimuli occurs when the duration between successive pulses of light is reduced to a certain value, this value being a function of the illumination and viewing conditions. The findings described in this report show that fusion is determined not only by the duration between successive stimuli but also by the temporal pattern of successive stimuli.

Most studies of visual flicker are made with intermittent photic stimuli of equal duration repeated serially (Fig. 1, *a* and *b*). Although such stimuli are usually described in terms of their frequency, the reciprocal of frequency, or period, may also be used for this purpose. As the frequency of an intermittent light is increased (that is, as its period is decreased) the apparent rate of flicker increases until a point is reached at which the light ceases to flicker and appears steady. The frequency at which this fusion occurs is called the critical flicker frequency.

The critical flicker frequency is usually taken to be a measure of the temporal resolving power of the eye. One explanation of fusion (*1*) is that at the critical flicker frequency the excitatory effects of one stimulus persist until the arrival of the next stimulus, providing a constant excitation process. A second explanation of fusion (*2*) is that there is a refractory period after each stimulation. Any stimulus arriving during this refractory period will fail to result in excitation. A third explanation (*3*) involves the threshold of brightness discrimination. Stimulation by a flash of light raises the excitation level to a certain value. When the light is turned off, the level of excitation starts to decline. The onset of the next flash of light raises the excitation level to its previous value. At the critical flicker frequency, successive pulses of light occur so rapidly that the excitation level fluctuates between limits which are not detectably different in brightness. All three explanations assume that for given viewing conditions fusion will occur whenever the duration between successive stimuli is less than a certain value.

Recently we have been studying visual flicker with intermittent photic stimuli of unequal periods (Fig. 1, *c*). Note that the train of pulses shown in *c* does not represent the mixing of two independent frequencies. With such stimuli flicker may be obtained even though the duration of each of the alternating periods is less than the duration of that period which, when repeated serially, is seen as fused. For example, if both *a* and *b* in Fig. 1 are above the critical flicker frequency, when alternated serially, as in *c* of Fig. 1, they may appear to flicker. The purpose of this report is to present data on the fusion contour for intermittent photic stimuli of alternating duration (*4*).

"On" and "off" triggers generated by four variable-frequency oscillators produced electric square waves which were the input to a Sylvania R1131C glow-modulator tube. The rise and decay time of the tube is approximately 20 μsec. The four oscillators were set so that two of them determined the "on" time and the "off" time, respectively, of one period; the remaining two determined the "on" and "off" times of the second period. They were then connected in series to produce intermittent electric square waves composed of two alternating periods (as in *c* of Fig. 1). Either of the two periods could be varied independently of the other. Within each period, "on" time always equaled "off" time. The glow-modulator tube illuminated a spot which subtended ½° of visual angle. The luminance of the test spot was approximately 1800 millilamberts (mlam); the luminance of the surround was approximately 22 mlam. All observations were made monocularly.

Measurements were obtained in the following manner: One period (*A*) was set at a fixed value while the other period (*B*) was varied by the observer until a fusion point was obtained. Measurements were first made with *A* set to a value of 2 msec. In successive measurements, the period of *A* was increased in 2-msec steps until it reached a value for which no further fusion points could be found.

Figure 2 shows the data for two subjects. The coordinates of Fig. 2 have been labeled in terms of both period and frequency. The frequency label has reference only to the frequency which either period would have if it were pre-

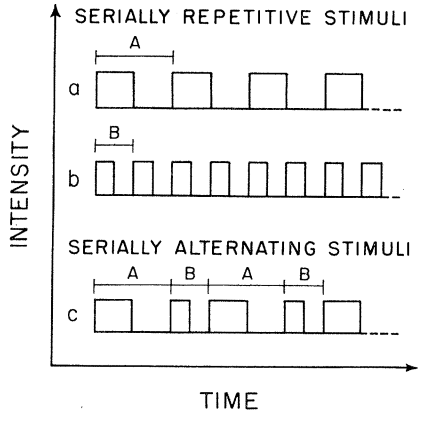


Fig. 1. Schematic representation of intermittent photic stimuli used in studies of visual flicker.

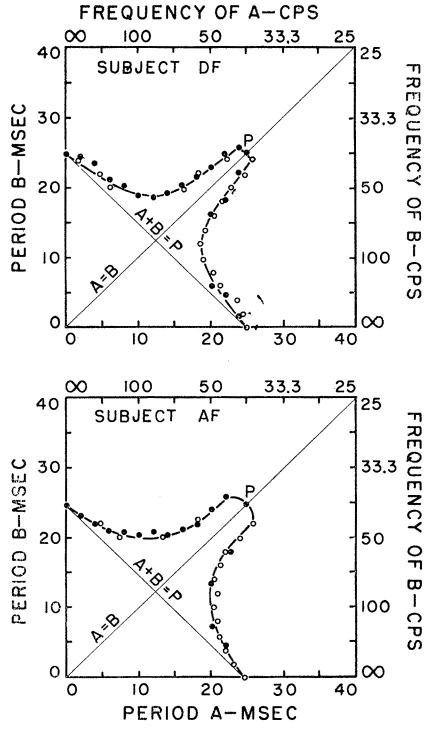


Fig. 2. Fusion as a function of the duration of alternate periods of intermittent photic stimulation. The solid circles are fusion points obtained by varying the period of *B*, with *A* constant. These points are based on ten observations per observer. The open circles are their mirror images.

sented as in *a* or *b* of Fig. 1. Each circle represents the values of two periods, *A* and *B*, which when alternated serially appear fused. Since the labeling of either period in such a combination is arbitrary, the circles below the diagonal line $A = B$ are mirror images of those above the line, and vice versa. The solid circles in Fig. 2 represent the fusion points obtained by varying period *B* while the period of *A* remained constant. These points are based on ten observations per observer. The open circles are the mirror images of the solid circles.

The fusion contour formed by passing a curve through all the points contains the traditional critical flicker frequency at three points: where it intersects the line $A = B$ and at its intersections with the two axes. All other points on the contour are for combinations of two periods of unequal duration. All combinations of *A* and *B* within the area bounded by the contour and the two axes appear as fused. All combinations of *A* and *B* which lie outside this area appear to flicker.

An examination of Fig. 2 shows that for some values of one period there are three values of the other which lie at a transition point between fusion and flicker. Thus, fusion may be reduced to flicker by increasing the average rate of stimulation—that is, by decreasing the duration of alternate periods. Conversely, flicker may be reduced to fusion by decreasing the average rate of stimulation —that is, by increasing the duration of alternate periods.

Note that no point on the contour falls below the line $A + B = P$, where *P* is the period associated with the critical flicker frequency. Even though flicker may be perceived when the period of each alternating pulse is less than *P*, the duration *P* appears to be a limiting factor for the temporal resolution of intermittent photic stimuli.

It is clear that the fusion of an intermittent light source cannot be explained solely in terms of a minimum duration between periods of stimulation for given illumination and viewing conditions. Fusion is rather a complex function of the temporal pattern of successive stimuli.

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29 September 1958

## Continuous Protein Synthesis in Nuclei, Shown by Radioautography with $H^3$-Labeled Amino Acids

*Abstract.* Radioautographic investigation of the cell nuclei of adult mice after injection of leucine-$H^3$, methionine-$H^3$, or glycine-$H^3$ shows a high uptake of tritium by chromatin material but not by nucleoli. It is concluded that protein synthesis occurs continuously within nuclear chromatin.

Radioautographs of tissues of rats given methionine-$S^{35}$ revealed that this amino acid is continuously being incorporated into proteins in all cells (*1*). Both nucleus and cytoplasm are involved in amino-acid incorporation (*2*), and, within the nucleus, a major role has been attributed to the nucleolus (*3*). However, this conclusion was based on investigations of large, but perhaps nonrepresentative, cells (starfish and amphibian ovocytes and diptera salivary-gland cells) from animals given amino acids labeled with $C^{14}$ or $S^{35}$. Since the high β-ray energy of these isotopes does not allow good radioautographic resolution but the low energy of tritium ($H^3$) does, tissues from mice injected with either leucine-, methionine-, or glycine-$H^3$ were investigated (*4*).

Thirty adult male C3H mice (26 to 32 g) were divided into three equal groups. The animals of each group received a single subcutaneous injection (5 μc/g of body weight) of one of the three amino acids: DL-leucine-4,5-$H^3$ (150 mc/mmole), DL-methionine-methyl-$H^3$ (7.2 mc/mmole), and glycine-2-$H^3$ (13 mc/mmole). Two animals of each group were sacrificed at 0.5, 4, and 35 hours and at 7 and 45 days. The tissues were fixed in Bouin's fixing fluid and processed through dioxan for histology; thus, free amino acids were removed (*1*). Six-micron sections stained with hematoxylin-eosin were radioautographed (*5*). Briefly stated, radioactivity distribution was similar with the three amino acids, and, in confirmation of previous observations with methionine-$S^{35}$ (*1*), radioautographic reactions were found not only over cells elaborating protein secretions (pancreas, thyroid, and so on) or undergoing renewal (hemopoietic organs, intestinal crypts, and so on) but over all other cells inves-

Table 1. Grain count over cytoplasm, nucleus, and nucleolus after injection of tritium-labeled leucine, methionine, or glycine in adult mice.

| Amino acid | Time interval (hr) | Grain count (per 4 $\mu^2$) | | |
|---|---|---|---|---|
| | | Cytoplasm | Nucleus | Nucleolus |
| | *Pyramidal cell (cerebrum)* | | | |
| Leucine-$H^3$ | 0.5 | 2.53 | 1.80 | 0 |
| Leucine-$H^3$ | 4 | 1.96 | 1.60 | 0.03 |
| Leucine-$H^3$ | 35 | 1.73 | 1.52 | 0.15 |
| Leucine-$H^3$ | 168 | 0.77 | 0.52 | 0 |
| Leucine-$H^3$ | 1080 | 0.31 | 0.17 | 0 |
| Methionine-$H^3$ | 0.5 | 0.23 | 0.15 | 0.03 |
| Methionine-$H^3$ | 4 | 0.97 | 0.63 | 0 |
| Methionine-$H^3$ | 35 | 0.43 | 0.62 | 0.16 |
| Methionine-$H^3$ | 168 | 0.07 | 0.06 | 0.01 |
| Methionine-$H^3$ | 1080 | 0.07 | 0.04 | 0 |
| Glycine-$H^3$ | 0.5 | 0.18 | 0.12 | 0 |
| Glycine-$H^3$ | 4 | 1.10 | 0.79 | 0.03 |
| Glycine-$H^3$ | 35 | 0.80 | 0.90 | 0 |
| Glycine-$H^3$ | 168 | 0.13 | 0.07 | 0.03 |
| Glycine-$H^3$ | 1080 | 0.13 | 0.03 | 0 |
| | *Purkinje cell (cerebellum)* | | | |
| Leucine-$H^3$ | 0.5 | 2.18 | 1.95 | 0.08 |
| Leucine-$H^3$ | 4 | 2.60 | 1.83 | 0.03 |
| Methionine-$H^3$ | 35 | 1.07 | 1.27 | 0 |
| | *Liver cell* | | | |
| Leucine-$H^3$ | 35 | 1.77 | 1.08 | 0 |
| Methionine-$H^3$ | 35 | 1.30 | 1.17 | 0 |
| | *Sertoli cell (testis)* | | | |
| Leucine-$H^3$ | 35 | 1.05 | 0.84 | 0 |
| | *Spermatocyte (testis)* | | | |
| Leucine-$H^3$ | 35 | 1.33 | 1.53 | 0.04 |