weeks, all surviving animals had liver neoplasm.

These results would indicate that, when as few as 50 tumor cells are injected intraportally and no growth is observed in the liver for at least as long as 3 months, it cannot be assumed that (i) the animal has been able to "cope" with these few cells, (ii) an "insufficient" number for growths has been inoculated, (iii) the cells did not "lodge" in the liver, or (iv) the cells were not viable. It would seem that they did remain in the liver in a viable but dormant state until triggered into growth by some factor or factors still to be elucidated but in all probability associated with surgical trauma with its attendant metabolic alterations. The mechanism involved in under study. It is suggested that cancer cells, alive to begin with, may be enduringly capable of growth if conditions are favorable (*6*).

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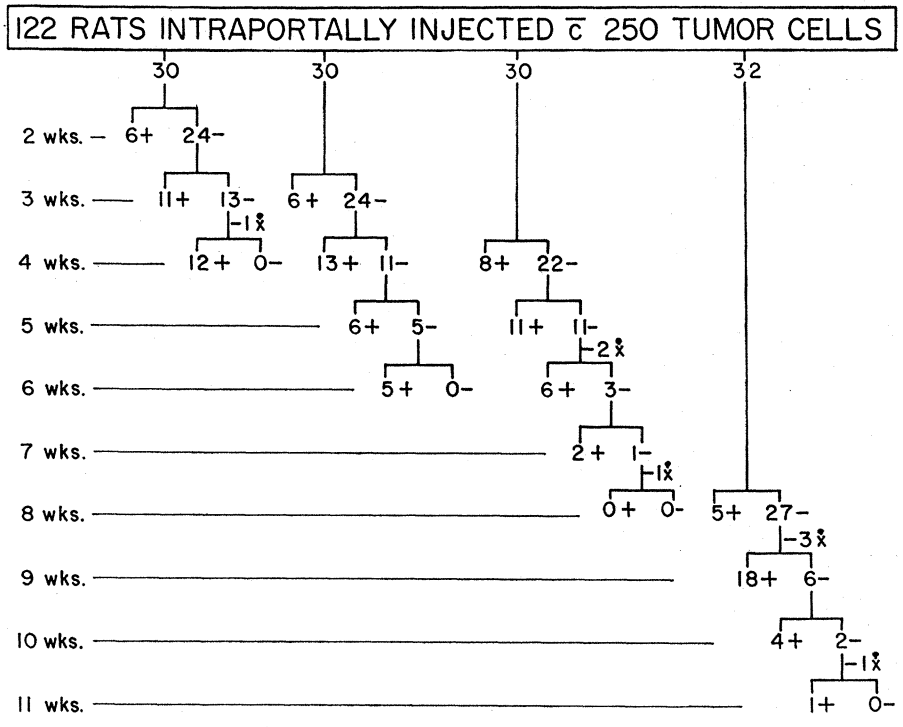

Fig. 1. Effect of repeated laparotomy upon the incidence of hepatic tumor growth following intraportal injection of 250 tumor cells: +, rats demonstrating liver tumor; —, rats free of hepatic tumor; *ẋ*, nonsurvivors.

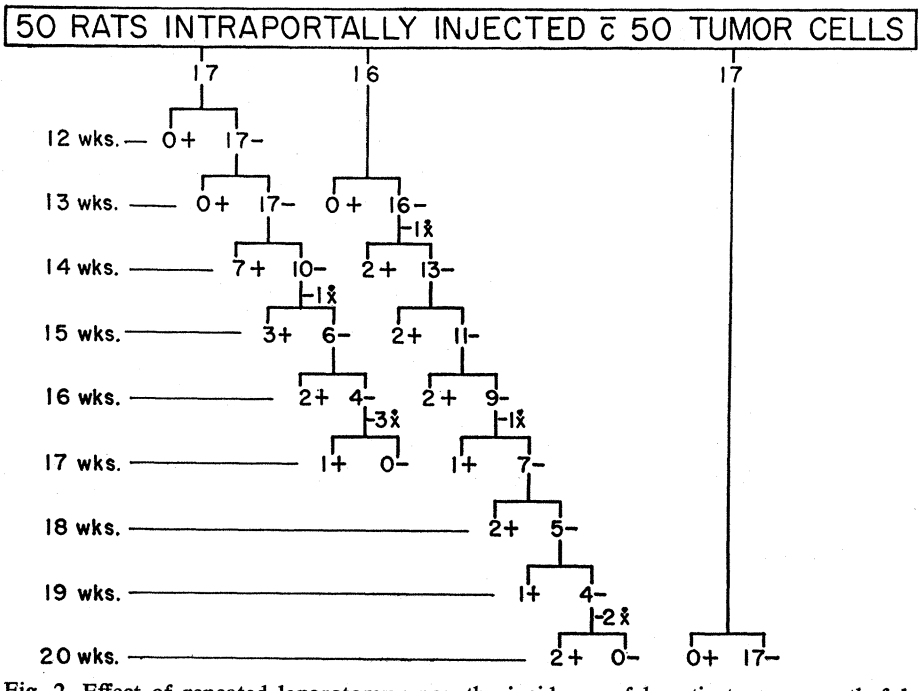

Fig. 2. Effect of repeated laparotomy upon the incidence of hepatic tumor growth following intraportal injection of 50 tumor cells: +, rats demonstrating liver tumor; —, rats free of hepatic tumor; *ẋ*, nonsurvivors.

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Fusion of Complex Flicker

Abstract. Brown and Forsyth have recently performed a flicker-fusion experiment showing clearly that there is more to flicker than meets the eye. This report presents an analysis of their results which indicates the likelihood that at fusion threshold all but one of the Fourier components of their presentation are imperceptible.

Brown and Forsyth have reported on a very interesting flicker-fusion experiment (*1*). They adopted a presentation extremely well suited to probing the visual processes taking place within a single cycle of flicker. A pulse $(A/2)$ msec long was followed by $(A/2)$ msec of darkness. Succeeding this was another pulse $(B/2)$ msec long, followed by $(B/2)$ msec of darkness. This sequence was continuously repeated. Those values of A and B which gave flicker fusion were then plotted, B against A (Fig. 2 of their report).

This report proposes an explanation of Brown and Forsyth's results and derives some implications from them. The explanation is based on a consideration of the Fourier components of their pulse sequence and on the explicit assumption that the subject discerns any one component at an amplitude which is independent of the presence of other components below threshold amplitude.

This treatment of stimulus components is suggested strongly by the results of de Lange (*2*) and of others cited by him. He presented a spot of constant average luminance modulated in various complex waveforms. For

every waveform, a plot of the amplitude a of the first Fourier component against fusion frequency f gave the same curve. If the higher, subthreshold components had had any influence on a, the different waveforms should have given at least slightly different curves.

The curves given by de Lange approach the form

$$a = kf^q \tag{1}$$

for a above one-tenth of the average luminance. The method for determining the proportionality constant k is given below. The exponent q appears to lie between 3 and 5, depending on the average luminance and on the observer.

The hypothesis already adduced is that Eq. 1 holds for any Fourier harmonic component of the presentation if that component is the only one at or above threshold. In the waveforms used by de Lange and others, the first Fourier component had the greatest amplitude. It is not surprising that the higher components did not seem to affect the results, especially in view of the strong frequency dependence noted above. However, in the complex stimulus presentation used by Brown and Forsyth, the first component may have too small an amplitude to be perceptible, while the second component may be quite perceptible.

It may be seen that Eq. 1 leads to curves of B versus A at fusion very much like Brown and Forsyth's. If only the nth Fourier component is at fusion threshold,

$$a_n = k(f_n)^q = k\left(\frac{n}{A+B}\right)^q \tag{2}$$

We have taken $A + B$ as the fundamental period of Brown and Forsyth's presentation at flicker fusion. The value of k may be found from Eq. 1: For the particular case of a pure square wave presentation of average luminance L, the amplitude of the first Fourier component is $4L/\pi$, and the fusion frequency may be written as $1/P$, to use Brown and Forsyth's symbol. Then $k = (4L/\pi)P^q$. Substituting in Eq. 2, we obtain

$$a_n = \frac{4L}{\pi}\left(\frac{nP}{A+B}\right)^q \tag{3}$$

For the general case $A \neq B$, the amplitude of the first Fourier component is

$$a_1 = \frac{4L}{\pi}\cos\pi\frac{A}{A+B} \tag{4}$$

and that of the second,

$$a_2 = \frac{4L}{\pi}\sin^2\pi\frac{A}{A+B} \tag{5}$$

The relation between A and B for fusion of the first component may therefore be found by substituting Eq. 4 in Eq. 3, obtaining

$$\cos\pi\frac{A}{A+B} = \left(\frac{P}{A+B}\right)^q \tag{6}$$

and for fusion of the second component, by substituting from Eqs. 5 and 3,

$$\sin^2\pi\frac{A}{A+B} = \left(\frac{2P}{A+B}\right)^q \tag{7}$$

Equations 6 and 7 are much more readily plotted as relations between $(A+B)/P$ and $A/(A+B)$ than as relations between A and B. The appropriate coordinate system is indicated in Fig. 1. In this system $(A+B)/P$ may be plotted against $A/(A+B)$ according to Eqs. 6 and 7 so as to give B/P versus A/P on Brown and Forsyth's axes. Trial values of $q = 3$ and 4 were used in plotting Eq. 6 (the solid curves). The value $q = 4$ fits Brown and Forsyth's data best. It is used in plotting Eq. 7 (the dashed curve). The reflections of the curves in the $A = B$ line are not plotted, to simplify an already crowded figure.

The two $q = 4$ curves fit the data accurately enough to suggest that, as was assumed, responses to different flicker components are largely independent. It is not within the scope of this general analysis to explain the small, but rather consistent, differences between subjects. As well as one can tell from the data, in the fusion region the observer responds independently to whichever component is above threshold.

At the present time more experiments illustrating the visual process postulated here might be of interest. One simple modification of Brown and Forsyth's approach suggests itself—namely, to add luminance modulated sinusoidally at an amplitude equal to that given by Eq. 4, and to adjust its phase for cancellation of the flicker. This would test the sensitivity of the eye to this component and would also allow one to follow the curve represented by Eq. 7 to more divergent values of A and B. The necessary signal voltage could be obtained

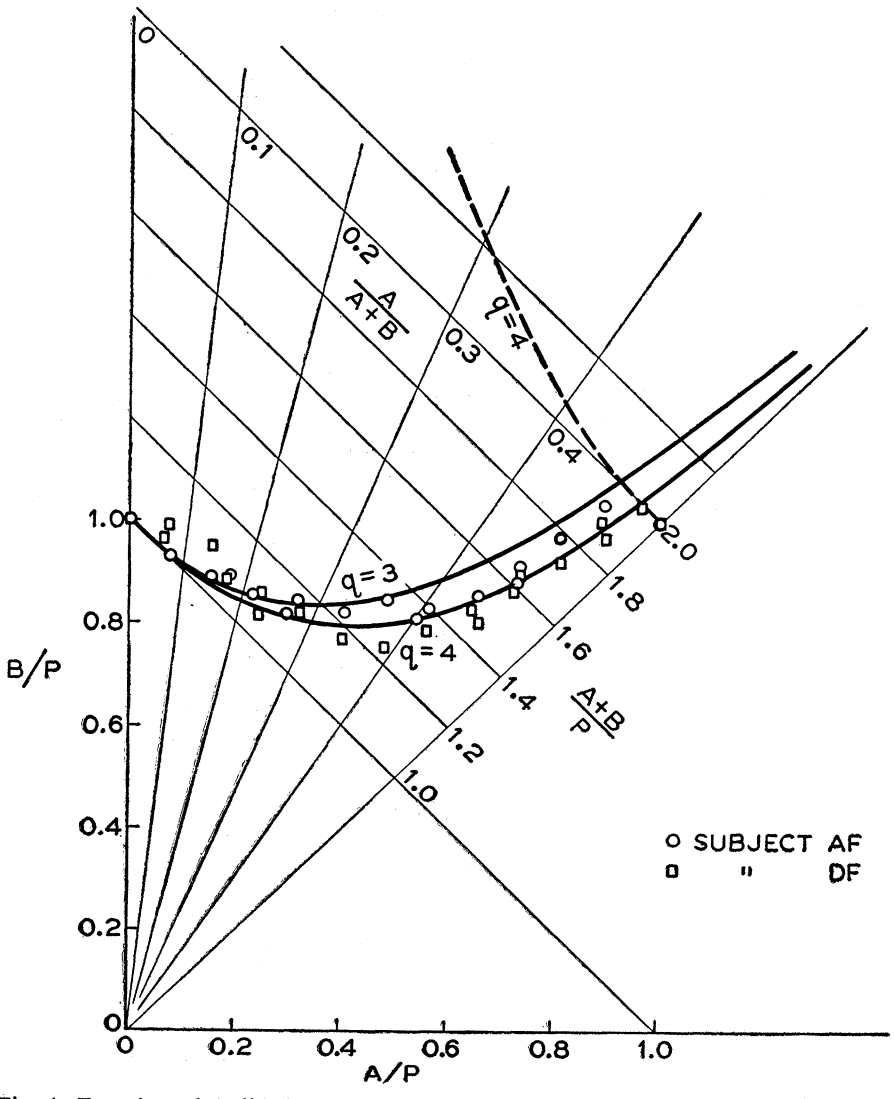

Fig. 1. Equations 6 (solid line) and 7 (dashed line) plotted on axes like Brown and Forsyth's. The experimental points are taken from Brown and Forsyth's report by optical projection and tracing.

from the stimulus control voltage itself and amplified, and its phase could be controlled without change of amplitude by a circuit such as may be found in *Waveforms* (*3*).

One change in Brown and Forsyth's sequence may yet reveal an intracycle effect, namely, the interchange of light and dark phases. The "Metacontrast" (*4*) effect may enhance flicker enough to depress the curves noticeably in the region of small values of A. For best discrimination of change of threshold with interchange of light and dark phases, the presentation could be switched back and forth between the two modes for each setting of A. The difference would certainly be slight, but might be noticeable, especially at lower luminances and frequencies. If noticeable, the effect would qualify the hypothesis of independence of threshold of Fourier components.

Finally, it is possible that an observer could satisfy himself rather directly that he is responding to one or the other of the two Fourier components, for values of A and B near the intersection of the two curves. If B were set slightly greater than P, there should be two nearly equal values of A for which flicker is observed. The flicker frequencies should differ by a factor of 2, very nearly—a difference large enough to be discriminable.

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4 May 1959

Competitive Antagonism between Kinetin and 8-Azaguanine in Polytoma uvella

Abstract. The enhancement of mitotic activity by kinetin is proportional to the dosage from 0.003 to 0.03 μg/ml. This effect is competitively antagonized by 8-azaguanine. The suppression can be reversed by a fresh supply of kinetin.

Kinetin (6-furfurylaminopurine) has been described as a substance which induces and stimulates mitotic divisions in tobacco pith tissue cultures (*1*). Furthermore, it could be shown that, in such kinetin-treated cells, doubling of deoxyribonucleic acid occurs prior to mitosis (*2*). In the course of studies on virulence changes in *Pneumococcus* and *Brucella* it was found that either deoxyribonucleic acid breakdown products or kinetin caused a selective multiplication of virulent cells within a mixed population (*3*). Stimulation by kinetin has been demonstrated in several other biological systems, such as Yoshida sarcoma cells (*4*), *Paramecium caudatum* (*5*), *Euglena gracilis, Ochromonas malhamensis,* yeasts (*6*), *Polytoma uvella* (*7*), and various plant tissues (*8*).

Since 8-azaguanine is known to inhibit mitotic divisions (*9*), this purine derivative was tested for an antagonistic effect on the mitosis-stimulating activity of kinetin (*10*). *Polytoma uvella* (WH strain) was chosen as a test organism. This phytoflagellate is a representative of the chlorophyll-free Volvocales; details of its culture and life cycle have been described elsewhere (*11, 12*). The test organism becomes multinucleate prior to cell division; experimental data were therefore collected as nuclear counts. Enhancement of mitosis is expressed as percentage increase of nuclei in treated cultures over untreated cultures after 18 to 22 hours, when the kinetin effect is most pronounced; that is, a 20-hour culture contained 610,000 nuclei per milliliter, while the corresponding kinetin-supplemented culture (0.06 μg/ml) had an increased nuclear count of 1.340 million per milliliter. This is expressed as 120 percent enhancement. Within the range from 0.003 to 0.03 μg/ml, the kinetin effect is proportional to the dosage (*11*). In all the experiments described, 8-azaguanine was used at a level of 10 μg/ml or less; at these levels it is noninhibitory with respect to *Polytoma.*

In the experiments performed to demonstrate antagonism, 8-azaguanine and kinetin were tested simultaneously in varying dosages (Table 1). It may be seen that the degree of suppression of kinetin enhancement depends on both the level of kinetin and the level of azaguanine. These findings indicate a competitive antagonism.

We have also been able to demonstrate the reversibility of the antagonistic effect. A set of cultures grown for 18 hours with 0.3 μg of azaguanine and 0.03 μg of kinetin per milliliter exhibits a suppression of 48 percent. Upon the addition of a fresh kinetin supplement (0.1 μg/ml), the suppression is markedly relieved by the 20th hour and is no longer evident at the 22nd hour.

Table 1. Suppression of kinetin effect by various dosages of 8-azaguanine.

Item	Percentage at various concentrations of kinetin					Av. of suppression (%)
	0.003 μg/ml	0.006 μg/ml	0.01 μg/ml	0.02 μg/ml	0.03 μg/ml	
Effect	+45	+65	+80	+96	+115	
			*Azaguanine + kinetin (1:1)**			
Reduced effect	+32	+50	+59	+75	+93	
Suppression	29	23	26	23	19	24
			Azaguanine + kinetin (10:1)			
Reduced effect	+20	+34	+39	+45	+60	
Suppression	56	48	51	53	48	51
			Azaguanine + kinetin (100:1)			
Reduced effect	+ 8†	+15	+18	+25	+32	
Suppression	(82)	77	78	74	72	75

* The corresponding molecular ratios are 1.5:1; 15:1; 150:1.
† Enhancement of 10 percent or less is of no statistical significance.

The fate of 8-azaguanine in living systems has been the topic of numerous studies, recently reviewed by Markham (*13*). This antimetabolite may replace guanine in nucleosides, in nucleotides, and in nucleic acids, or it may block a guanine-containing coenzyme system. Bergmann and Kwietny (*14*) have recently shown that kinetin is converted into 2,8-dihydroxy-kinetin by xanthine oxidase, and they discuss the possibility that "kinetin may react similarly to adenine in other enzyme systems." However, Kalckar *et al.* (*15*) found that inhibitors of xanthine oxidases did not influence the kinetin effect. We believe that kinetin is converted enzymatically to a still unidentified product, as is indicated by the observed competitive antagonism between kinetin and 8-azaguanine.

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