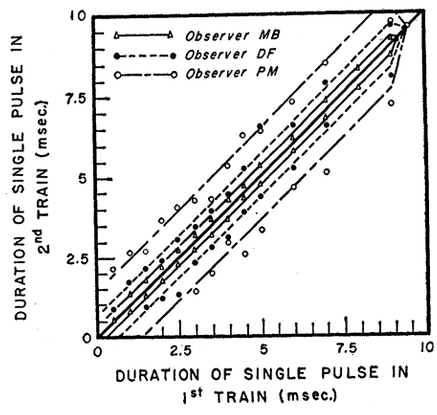

Fig. 3. Duration of a pulse in the second train (Y) as a function of duration of a pulse in first train (X) for fusion. Points represent thresholds from Forsyth and Brown's experiment. Straight lines on both sides of diagonal are predicted values of Y at fusion. (In Forsyth and Brown's report $2X$ and $2Y$ were plotted on the axes.)

rigorous treatment involves exact knowledge of P and R and requires using other families as well. For example, for $X = 8⅓$ msec, the smallest value of $2PX \geqq 40$ msec is 50; hence, the family for $T = 100$ could be used at $X = 8⅓$ msec. As can be determined from Fig. 2A the slope of the a_1/L versus Y function with $T = 100$ is only slightly different than for the $T = 80$ msec family, and if we assumed the same criterion amplitude at $T = 100$ as at $T = 80$, the predicted points would be changed by an amount that is well within the noise level of the present type of experiment. However, as T is changed, $f_1 = 1/T$ is changed, and as shown by de Lange's data (*3*), the criterion amplitude would also be changed. These variations are, however, also not large in the region of present interest. They would introduce only further slight changes in prediction.

We have so far assumed $2PX = 2RY$. Forsyth and Brown's description suggests that they did not deviate widely from this condition. However, briefly consider what happens when $2PX \neq 2RY$. Assuming X and $2PX$ constant (for example, $X = 5$ and $2PX = 40$ msec as in Fig. 2B) we note that for a particular value of $2RY$ larger than 40 msec, A_1/L increases as Y deviates from X in either direction; also, for a particular value of Y, as R increases beyond the region in which $2RY = 40$, A_1/L decreases monotonically. For near-threshold values of Y (for example, 4 and 6 msec in Fig. 2B) this decrease is slow. Nevertheless, deviations from the condition $2PX = 2RY$ could be involved in the small differences between the data and predicted values. Since the experimenters did not use values of $2RY$ smaller than 40 msec, deviations from $2PX = 2RY$ would have resulted in a small systematic bias in the direction of larger differences between Y and X at threshold. Such a bias leads to predictions of higher criterion amplitudes for the first component than were in fact employed by the subject and is very probably the reason that two of the criterion amplitudes inferred above were slightly higher than those reported by de Lange.

The particular interest in Forsyth and Brown's results stems from the appearance of flicker when each of two alternating trains has a repetition rate far above the usual values of critical flicker frequency. The present treatment makes it clear that the observer in this situation discriminates variation of a single low frequency component against a background of high frequency components which are steady as far as the observer's visual system is concerned. While the highest repetition rate used within a single train was 1000 cy/sec, it can be predicted from Eq. 4 and Fig. 2A that there should be no upper limit whatever. For any value of P it should be possible to find small enough values of R so that the first component will be above the flicker threshold; for high P values (greater than 1000 or 2000 cy/sec), however, it is not expected that any values of R larger than P would result in the appearance of flicker.

Some earlier experiments of Brown and Forsyth (*5*) had been analyzed by Levinson (*6*). In these experiments, two trains, each with a single pulse of different duration were alternated and fusion contours plotted for the durations of the two pulses. The case treated in the present report is thus a general case of that situation and reduces to it when $P = R = 1$. While the present type of linear frequency analysis may be expected to hold for any stimulus in which the amplitudes of all but one of the frequency components are sufficiently below threshold, when two or more components are close to threshold it is not certain that simple superposition will be applicable; it has in fact been shown that it does not hold for one such set of conditions (*7*), (*8*).

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References and Notes

1. D. M. Forsyth and C. R. Brown, *Science* **134**, 612 (1961).
2. E. Ives, *J. Opt. Soc. Am.* **6**, 254 (1922); P. W. Cobb, *ibid.* **24**, 91 (1934).
3. H. de Lange, *ibid.* **44**, 380 (1954); **48**, 777, 784 (1958); **51**, 415 (1961).
4. The dashed lines in Fig. 2A and 2B were obtained for each subject as follows: The average deviation of the observed Y values from the line $Y=X$ in Fig. 3 was calculated. This value was plotted in Fig. 2A as a horizontal distance from the point $Y=X$ on one curve of the family; the vertical erected at the plotted point crossed the same a_1/L versus Y curve at an a_1/L value that was then assumed to be the criterion amplitude. Since f_1 is the same for all members of the family it was assumed that the same criterion amplitude was required for fusion for all of them.
5. C. R. Brown and D. M. Forsyth, *Science* **129**, 390 (1959).
6. J. Levinson, *ibid.* **130**, 919 (1959).
7. ———, *ibid.* **131**, 1438 (1960).
8. This work was supported by grant G-18120 from the National Science Foundation.

6 October 1961

Organ Cultures of Newborn Rabbit Skin Affected by Rabbit Papilloma Virus

Abstract. Small fragments of newborn rabbit skin were exposed to papilloma virus and transferred to tissue culture. Control cultures were treated the same way, but without addition of the virus. These cultures were then followed for 4 weeks. During the first ten days, the number of epidermal elements declined gradually in both test and control cultures. From the 10th day on, however, a marked proliferation of the remaining epidermal cells was observed in cultures exposed to the virus, whereas no further changes were noticed in the control cultures.

Dawe and Law infected organ cultures of mouse salivary glands with polyoma virus and noticed a proliferation of the glandular epithelium in the infected cultures (*1*). Lasfargues and colleagues found evidence for the multiplication of the mouse mammary tumor virus in organ cultures of mouse mammary epithelium, but obtained no results with roller tube cultures of pure epithelial cells (*2*). The experiments described in this report (*3*) were designed to investigate the effect of the rabbit papilloma virus (Shope) on organ cultures of rabbit skin.

The skin of 1- to 5-day-old rabbits (*4*) was cut into small fragments about 3 mm^2. The fragments were dipped in a suspension of virus obtained from cell-free extracts from warts of cottontail rabbits (*5*), and previously tested for potency in the skin of domestic rabbits. The skin fragments were placed, keratin side up, in a semisolid medium consisting of medium 199 (*6*) with 0.5 percent

agar (*7*). This medium covered the bottom of a small plastic petri dish. The imbedded skin fragments were then covered with 1 ml of a fluid medium consisting of 60 percent Puck's medium (*6*), 25 percent fetal bovine serum (*6*), 15 percent rabbit serum (*4*), and 100 units of penicillin and streptomycin per milliliter. Then 0.1 ml of virus suspension was added to the culture. Uninfected fragments were grown for controls.

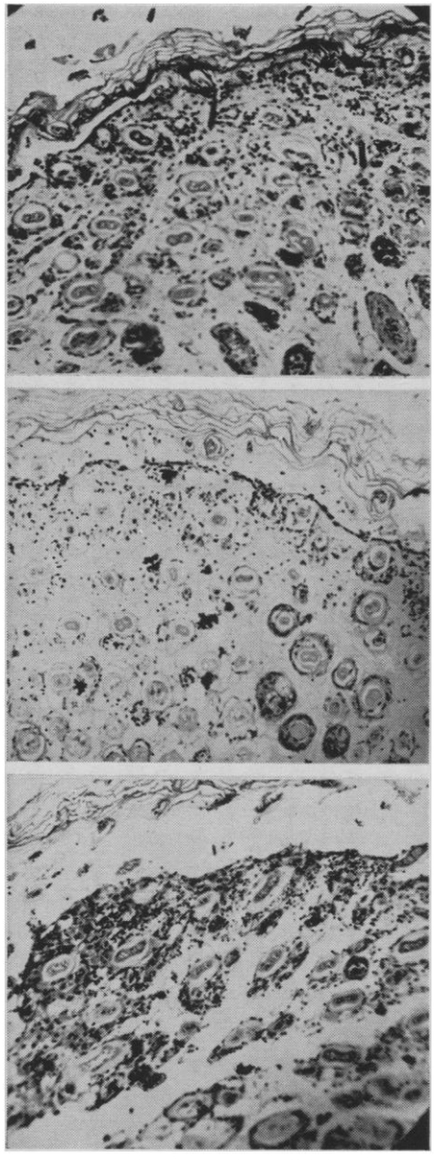

Fig. 1. Sections of the cultures at various stages. Hematoxylin and eosin, about × 90. (Top) Control culture after 5 days. The aspect is still very much that of noncultured rabbit skin, except for the separation between the keratin and the basal layer. At this stage the virus-infected cultures have the same aspect as the control cultures. (Middle) Control culture after 20 days. A single layer of epidermal cells covers the surface of the fragment. Note also atrophy of the epidermal elements of the hairfollicles. (Bottom) Virus infected culture after 20 days. The epidermal cells have invaded the whole fragment.

All cultures were grown for 30 days at 37°C in a humidified atmosphere of 5 percent CO_2 in air. The fluid phase of the medium was changed every 4 days, but the fragments of skin were left imbedded. At intervals of 3 or 4 days, some control and virus-treated fragments were taken out for histological examination. Tissues were fixed with buffered formalin and stained with hematoxylin and eosin. Serial sections were then made of each fragment examined.

Three series of experiments were run, each time with virus from different papillomas. The evolution of both control and virus-infected cultures was the same until about the 10th day, and the number of epidermal cells gradually declined. In control cultures only a single layer of epidermal cells remained at the end of 30 days. These cells covered the upper surface of the fragment or, rarely, encased the whole fragment. The epidermal elements of the hair follicles also decreased, and none were found outside the follicles (Fig. 1, top and middle). In the virus-treated cultures, from the 10th day on, there was a marked proliferation of the epidermal cells, starting on the upper side of the fragment and gradually invading the whole fragment (Fig. 1, bottom). It was impossible to determine whether the proliferation had its origin in the basal layer, in the hair follicles, or in both. Nearly all virus-treated fragments were invaded by epidermal elements. In another experiment with adult rabbit skin, proliferation was not consistent, occurring only occasionally in some virus-infected cultures.

Apart from Coman, who found stimulation of cell growth in roller tube cultures of rabbit epithelial cells (*8*), there are, to my knowledge, no reports of any clear-cut effect of the rabbit papilloma virus *in vitro*. It is therefore significant that this virus, which has never shown any cytopathic effect in regular tissue culture, caused cell proliferation when applied to organ cultures. There may indeed be other tumor viruses which, when tested on monolayers of trypsinized cells, either do not show any effect and thus escape notice or cause mainly a cell destruction and hence have been classified as nontumor viruses. The results obtained by Dawe and Law with the polyoma virus, the findings of Lasfargues with the mouse mammary tumor virus, and our results with the rabbit papilloma agent show that, for the study of tumor agents, organ cultures offer an interesting alternative to monolayers of trypsinized cells. More especially, organ cultures of human tissues may offer an approach to investigate the possible neoplastic abilities of extracts from human tumor material, or agents already isolated from such material.

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References and Notes

1. C. J. Dawe and L. W. Law, *J. Natl. Cancer Inst.* **23**, 1157 (1959).
2. E. Y. Lasfargues, M. R. Murray, D. H. Moore, *Natl. Cancer Inst. Monogr. No. 4* (1960), p. 151.
3. This work was aided by a grant from the American Cancer Society.
4. Rockefeller Institute Colony.
5. Obtained through the kindness of Dr. Peyton Rous of the Rockefeller Institute.
6. Microbiological Associates.
7. Difco Laboratories.
8. D. R. Coman, *Cancer Research* **6**, 602 (1946).
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Effects of Increments and Decrements of Light on Neural Discharge Rate

Abstract. Neurons in the lateral geniculate nucleus of the monkey discharge impulses periodically under conditions of both dark and light adaptation. Brief intensity changes in one direction produce decreases in firing rate roughly proportional to the logarithm of the intensity; intensity changes in the other direction produce corresponding increases in firing in the same neuron.

The presence of a maintained neural discharge in cells in the visual system, regardless of whether the eye is being stimulated with light or not, has led to the view that the response to light must be a modulation of this maintained firing rate rather than the firing of a certain number of spikes (*1*). Nevertheless, the generally accepted view is that it is only the increase in firing rate from the maintained level which is carrying significant information about the stimulus conditions.

We have argued, on the basis of finding cells in the lateral geniculate nucleus of the monkey which show graded increases and decreases of firing rate depending on the wavelength of the light (*2*), that a single cell can carry information about two different (and