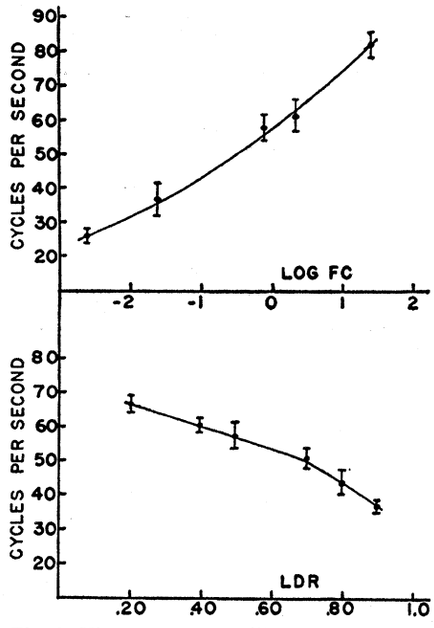

Fig. 2. The upper curve illustrates relationship of target brightness to critical flicker frequency at light-dark ratio (LDR) of .50. The lower curve illustrates relationship of LDR to the critical flicker frequency. Both curves are from the same monkey.

mon values were about 35 responses (both A and B) per reward, and about two rewards per minute.

The stepping relay that controlled flash rate was linked to a graphic recorder so that a continuous record of lever pressing behavior was obtained on a moving chart, calibrated directly in cycles per second for each range employed. Several somewhat arbitrary control procedures were established to determine whether the record being generated represented some form of oscillatory or random behavior or reflected attention to the flash rate by the monkey. These consisted of altering the range of frequencies available and introducing large changes in rate during a food interval. If the monkey produced a new stabilized record in agreement with that produced before the changes, the record was considered to be a reflection of the flicker fusion phenomenon. An example of such a record is seen in Fig. 1. These control procedures led to the rejection of from 20 to 40 percent of threshold records.

In order to compare results of this method with those obtained by different methods it was necessary to designate a critical flicker frequency threshold in cycles per second for a given monkey's performance under a particular set of target parameters. Reasoning that the end of an "A run," or increasing steps of flash rate, corresponded to the point when the target just became equivalent to *Sx* (by definition a fused light), I decided to use only those points for assigning threshold values. Acceptable sections of threshold record were analyzed for the range of those points (eliminating the one highest and lowest), and the midpoint of that range designated the critical flicker frequency, expressed to the nearest whole number of cycles per second.

Despite the rather crude method of assigning frequencies a useful degree of reliability has been obtained. Five of the six monkeys tested showed relatively stable critical flicker frequency thresholds (within 10 cy/sec) which did not change systematically with repeated testing. The most extensively studied animal has shown no improvement over more than 8 months of regular testing. The type of problem for which these procedures appear ideally suited involves the testing of thresholds at a number of values of some stimulus parameter, or following a threshold continuously as a function of some changing physiological variable. Examples of the former are shown in Fig. 2, which illustrates the effects of varying target brightness or light-dark ratio on the critical flicker frequency. Brightness was varied by means of neutral density filters in the light path, and light-dark ratio (more exactly, light to cycle ratio) was varied electronically, a compensated Talbot brightness of 0.8 ft-ca being used for all ratios.

The range of critical flicker frequencies under standard conditions (brightness 2.3 ft-ca, light-dark ratio = .50) observed in this series of monkeys has been wide, extending from just under 30 to about 61 cy/sec. Stability of threshold and wide individual differences have also been observed in a different series of 11 monkeys tested on a conventional "go no-go" type of discrimination (*5*). It appears that values of critical flicker frequency with the same target parameters have tended to be higher than those observed with the self-determination technique, but with a great deal of overlap. Consistent discrimination at very high rates (above 80 cy/sec) has been seen with the "go no-go" method but not with the self-determination method (*6*).

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References and Notes

1. E. Simonson and J. Brozek, *Physiol. Revs.* **32,** 349 (1952).
2. D. S. Blough, *Science* **121**, 703 (1955); *J. Exptl. Anal. Behav.* **1,** 1 (1958).
3. I am indebted to D. S. Blough for many helpful suggestions regarding the development of these procedures.
4. The light source consisted of a Sylvania R113C glow modulator tube driven by a square-wave generator allowing independent adjustment of frequency, duty cycle, and peak current. Light pulses were monitored by a GE 930 photocell and found to be rectangular with extremely brief rise and decay times. I am indebted to John T. Conrad for help in designing the flicker circuits. Light pulses were viewed by the unrestrained monkey in the dark through a 3-inch diameter ground-glass target at distances between 1 and 6 inches.
5. A description of the details of method and results is in preparation. The procedure was a replication in most features of that used by M. Mishkin and L. Weiskrantz [*J. Comp. and Physiol. Psychol.* **52**, 660 (1959)].
6. This research was supported by grant B2681 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service. A preliminary report was read at the Eastern Psychological Association meeting in April 1961.

23 January 1962

Fungilytic Activity of a Species of Verticillium

Abstract. A species of *Verticillium* arrested the growth of fungi by extracellular secretions on agar media. The lytic agent in the culture filtrates appeared to be thermolabile and was active in vitro on spores of *Puccinia graminis.* The possibility that the lytic agent is an enzyme has been investigated and purification studies are in progress.

Experiments with species of *Verticillium* have shown that these fungi produce an abundance of enzymes, among which pectin-splitting enzymes are most important (*1*). The discovery of these enzymes has constituted a major step forward in fundamental plant disease research, since it has been shown that the enzymes are responsible for wilt symptoms in susceptible plants.

A study has been made of the ability of *Verticillium* to suppress the growth of fungi, particularly by the production of antifungal enzymes. The most active *Verticillium* strain tested was isolated from a coffee plant disease (*2*).

It was observed that the *Verticillium* completely inhibited fungus growth in marked contrast with the corresponding control cultures without the *Verticillium.*

Direct attack by *Verticillium* sp. on spores and hyphae of *Hemileia vastatrix* has taken place in water. Washed spores of *Hemileia* were suspended in distilled water and incubated with an inoculum

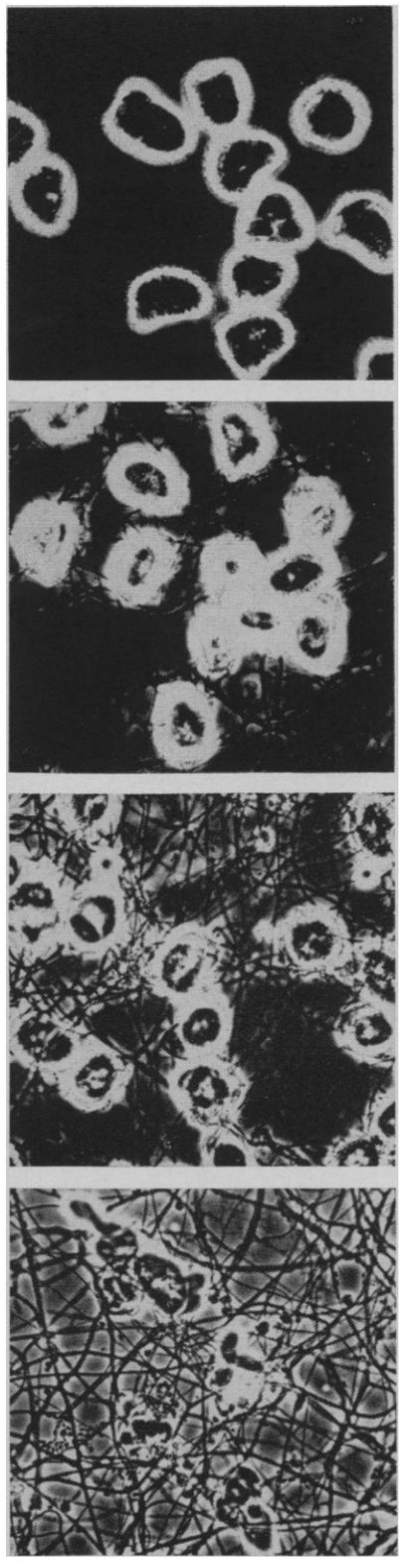

Fig. 1. *Hemileia vastatrix* spores: (top) before inoculation with *Verticillium* sp.; (upper middle) 2 days after inoculation, showing *Verticillium* beginning to attack rust spores; (lower middle) 10 days after inoculation with *Verticillium* sp.; (bottom) 2 weeks after inoculation, showing complete digestion of the *Hemileia* spores and full sporulation of *Verticillium*.

of *Verticillium* sp. The mold developed luxuriantly on the rust spores during the first 2 days. After about 2 weeks very few rust spores capable of taking up lactophenol blue were found, though sometimes it appeared that the walls of these spores had not been attacked (see Fig. 1).

The existence of a fungilytic secretion was demonstrated on solid media. One petri dish was inoculated with *Helminthosporium gramineum* and incubated. After 7 days, standardized colonies of *Verticillium* sp. were prepared on each plate and the incubation was continued. The plates were observed for 4 weeks and during this time a marked attack on the *Helminthosporium* mycelium was seen. The same kind of experiment was repeated with various other molds and in most experiments the *Verticillium* grew directly on the fungus mycelium.

Plates prepared with heat-inactivated rust spores were also inoculated with *Verticillium* and after incubation for 1 week each colony was surrounded by a clear zone, which suggested lysis of the test organisms. Presumably the same agent was responsible both for the partial lysis on agar media and for the direct attack on the rust spores in aqueous suspension.

The possibility that the lytic agent was an enzyme was investigated. One-milliliter portions of a suspension of spores or minute fungus colonies (previously thoroughly washed), were added to 4-ml portions of: (i) sterile water; (ii) filtrates of liquid *Verticillium* sp. cultures (glucose-asparagine yeast extract solution); (iii) heated culture filtrates. Triplicate tubes were incubated and samples were removed at intervals (up to 2 weeks) for microscopic examination. In the water tubes the contents of the rust spores were almost entirely stainable with lactophenol blue. The condition of the spores in the heated culture filtrates was essentially the same as in water. The spores in the unheated culture filtrates had almost completely lost their ability to take up the strain.

It seems from these results, and from those of the experiment in which strong direct attack has taken place on the test organisms in growing cultures or in water (that is, under conditions of nutrition unlikely to favor antimetabolite production), that some thermolabile lytic substance was produced by the isolated strain of *Verticillium*. This heat lability and the pattern of activity, suggest that the lytic substance is an enzyme.

Attempts to obtain a cell-free enzyme preparation were made with the culture filtrates. The filtrates were frozen and freeze-dried. The freeze-dried material could be stored at 5°C for several weeks, without loss of activity. Attempts to purify the lytic enzyme by procedures of protein fractionation are in progress.

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References

1. V. W. Cochrane, *Physiology of Fungi* (Wiley, New York, 1958), p. 115; D. C. Deese and M. A. Stahmann, *Univ. Wisconsin Agr. Expt. Sta. Ann. Rept. (1958)*.
2. J. A. Leal, *Nature*, in press.

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Effect of Acetylcholine on the Electrical Activity of Somatic Nerves of the Lobster

Abstract. The effects of acetylcholine (ACh) on axons of the walking leg of lobster have been investigated. In $10^{-2}M$ concentration the ester lowers the resting potential by approximately 7 mv within 10 minutes. Simultaneously, the spike height is decreased. Complete but reversible block ensues in 10 to 15 minutes. At lower concentrations essentially similar but smaller effects are observed. Choline chloride and sodium acetate, at concentrations of $10^{-2}M$, are without any effect. In conjunction with the evidence accumulated for the essential role of acteylcholine in the control of ion movements, the demonstration of the ability of acetylcholine to reversibly depolarize axons of somatic nerve fibers appears pertinent.

The failure of acetylcholine (ACh) to affect axonal conduction in contrast to its action on synaptic junctions is frequently cited as a major objection to Nachmansohn's theory of the essentiality of ACh in axonal conduction (*1*). Mono- and diquaternary nitrogen derivatives, such as ACh and curare, are lipid insoluble. Nachmansohn has explained their failure to affect axons by the existence of structural barriers which prevent lipid insoluble compounds from reaching the conducting membrane; this is in contrast to the relatively greater permeability of the axon to the lipid soluble tertiary nitrogen compounds, such as eserine and the local anesthetics closely related in