

This histological study revealed some of the minute details of the vector-virus-host relationship of the sweetpotato yellow dwarf virosis and demonstrated the precision of the surgical operation performed by this insect in its normal feeding operations. Other unreported evidence supports the hypothesis, which definitely applies to all whiteflies and aphids, that whenever a stylet is less than $3\ \mu$ in diameter penetration will be intercellular, because its physical size makes it too limber for direct penetration.

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Chemical Nature of Antheridogen-A, a Specific Inducer of the Male Sex Organ in Certain Fern Species

Abstract. Antheridogen-A has been shown to be a complex carboxylic acid. The carboxyl function is necessary for inductor activity since this activity disappears on esterification and reappears after hydrolysis of the ester.

Recently a substance was isolated from culture filtrates of the bracken fern *Pteridium aquilinum* which induces the formation of antheridia in gametophytes of the sensitive fern *Onoclea sensibilis* at a concentration of less than 1 part in 10,000,000,000 ($10^{-4}\ \mu\text{g/ml}$) (1). The present report describes what is known of the chemical nature of this antheridium-inducing factor, for which the trivial name antheridogen-A is proposed.

Since the material is present at a concentration of the order of $1\ \mu\text{g}$ or less per liter of culture filtrate, it has not yet been possible to produce enough material to analyze directly. The chemical constitution of antheridogen-A was studied indirectly by observing the effects of chemical manipulations on the purified concentrate prepared as previously described (1). Antheridogen-A dialyzes readily through cellophane

membranes, so it appears to have a low molecular weight. It is relatively stable in acid solution but is readily inactivated at pH above 7. It is also readily inactivated by oxidizing agents. During the course of isolation the compound behaved as a weak acid.

Antheridogen-A was found to have a distribution coefficient close to unity when partitioned between normal butanol and 5 percent ammonium acetate buffer of pH 6.65 (1). When partitioned between ethyl acetate, isoamyl acetate, tertiary amyl alcohol, or peroxide-free diethyl ether and McIlvaine buffers at various pH's, antheridogen-A distributed in a pattern which suggested it to have a pK_a of about 5.0.

The compound appears to be free of phosphorus. When an amount of material equivalent to $10\ \mu\text{g}$ was tested by the method of Hanes and Isherwood (2) no color was produced. All phosphate esters tested at this concentration gave strong positive tests. Ninhydrin tests on filter paper chromatograms of the material were consistently negative.

Treatment of a dry ethereal solution of antheridogen-A with excess diazomethane completely inactivated the material. When the methyl ester was refluxed with 5N hydrochloric acid for 3 hours, biological activity was restored.

Examination of the infrared absorption spectrum of antheridogen-A preparations revealed a well-defined maximum at $1700\ \text{cm}^{-1}$ which is consistent with a carboxyl functional group and perhaps suggests that the molecule contains an unsaturated carbon-carbon bond in the vicinity of the carboxyl group (3). The ease with which the inductor activity is lost by oxidation also suggests unsaturation. On the other hand, when the compound was treated with bromine in carbon tetrachloride solution for 4 hours, no loss of activity occurred.

The preceding indirect evidence indicates that antheridogen-A is a complex carboxylic acid. Furthermore, the carboxyl function is necessary for biological activity since this activity disappears on esterification and reappears after hydrolysis of the ester. A large number of naturally occurring carboxylic acids were tested for antheridium-inducing activity on *O. sensibilis*. None were found to have activity. It is of interest to note that certain long-chain aliphatic fatty acids produce a threefold increase in the potency of antheridogen-A preparations. This relationship may have some bearing on the mode of action of antheridogen-A and will be further investigated (4).

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References and Notes

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Maintenance of Avoidance Behavior under Temporally Defined Contingencies

Abstract. Operant behavior of rats was maintained at moderate frequencies by a temporally defined shock avoidance schedule. Progressive reductions in one of the temporal parameters of this schedule—the portion during which behavior may have consequences—yield an orderly response rate function which first rises to a maximum and then declines gradually to extinction.

A system for classifying schedules of reinforcement for operant behavior in terms of temporally defined parameters has recently been proposed by Schoenfeld, Cumming, and Hearst (1). This system, which provides a common dimensional framework for the specification of reinforcement schedules generally, defines two basic variables: t^p and t^a , time periods during which, respectively, reinforcement may be given and reinforcement is never given.

Conventionally, t^p and t^a are held constant and are alternated, and only the first response in t^p is reinforced. Some of the parameters of that system have been experimentally explored in a number of recent studies (2) in which positive reinforcement procedures were employed, but to date their effects have not been observed in avoidance conditioning contexts, where the occurrence of a given response prevents the presentation of an aversive stimulus. In the procedure adopted in the experiment reported here, an avoidance schedule lacking a warning stimulus (3) was cast in t^p , t^a terms, and the effects of systematically varying one of the temporally defined parameters were studied.

Four adult male hooded rats, all without prior experimental history, were exposed for 30 minutes daily to an avoidance conditioning schedule. The sound-resistant chamber in which all the animals worked was equipped with a lever and a grid floor through which electric shock (0.3 ma) could be delivered to the rat's feet. Depression of the lever activated counters and a cumulative recorder. Relays and timers established a temporally defined avoidance schedule composed of alternating t^a and t^p time periods, one following