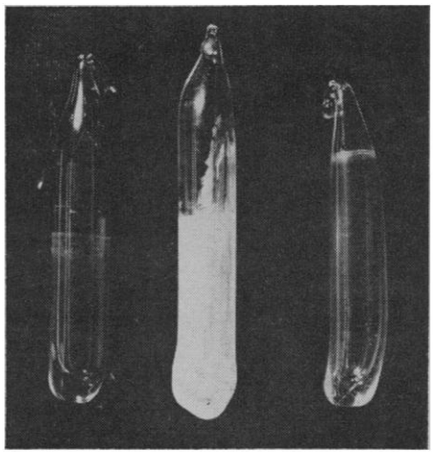

Fig. 2. Plummets immersed intermittently in synthetic solutions in an atmosphere of carbon dioxide (left to right): without enzyme, with enzyme, and with enzyme and inhibitor.

dioxide by bubbling the gas through them, the enzyme was not required in order for a deposit to form. These solutions presumably lost carbon dioxide until an optimum concentration of carbonate ions and *p*H favored precipitation.

However, when these synthetic solutions were not initially saturated with carbon dioxide and when the apparatus was enclosed in an atmosphere of carbon dioxide, the results (Fig. 2) were essentially similar to those obtained with boiled saliva: that is, no significant deposit was obtained unless carbonic anhydrase was added, and addition of sulfanilamide prevented formation of a deposit. These solutions contained also an organic buffer. The initial *p*H was 7.5 and no appreciable change took place accompanying the deposition.

In all cases the mineral depositions probably consisted of microcrystalline dahllite. By means of x-ray diffraction methods this fact was verified for three precipitates: the only deposit obtained from the second set of experiments with synthetic solutions (the one containing carbonic anhydrase) and the two significant deposits obtained with saliva (untreated saliva and boiled saliva to which carbonic anhydrase had been added). No evidence of the presence of a second crystalline phase was obtained (Fig. 3), but this does not necessarily prove that the nascent deposition has the crystal structure of an apatite. It merely proves that the stable inorganic substance in such a system is a carbonate hydroxyapatite (*3*), regardless of any inorganic precursor which might have existed (*4*).

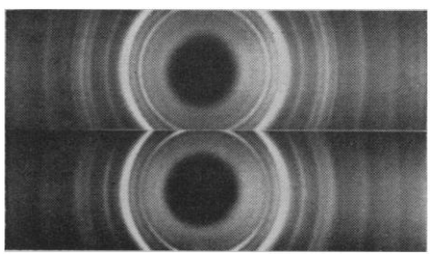

Fig. 3. Comparison of powder diffraction patterns of in vitro bone mineral precipitated from synthetic solution (top) and mineralized cartilage from the spinal column of a shark (bottom).

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3. The carbonate content of an apatite cannot necessarily be demonstrated by x-ray diffraction methods for microcrystalline materials, but it was confirmed by liberation of bubbles of gas during dissolution of the deposits in hydrochloric acid.
4. The specimen of mineralized connective tissue of a shark, which we find to be crystallochemically identical with bone, was supplied by Dr. Marshall R. Urist, Department of Surgery, University of California Medical Center, Los Angeles. The diffraction pattern indicates preferential orientation of the crystallites because the specimen was not powdered but was merely cut in the shape of a rod. This investigation was supported by a grant to the Ohio State University Research Foundation by the Procter & Gamble Co., Cincinnati.

26 September 1960

Relations between Whitefly and Sweetpotato Tissue in Transmission of Yellow Dwarf Virus

Abstract. One approach revealed the nature of the piercing-sucking-feeding mechanism of the abutilon whitefly, *Trialeurodes abutilonea,* a new vector of sweetpotato yellow dwarf virus. It showed the complete stylet from its origin in the rostrum to its termination in the plant's translocation stream. Another approach clearly delineated and confirmed the life cycle of the abutilon whitefly in relation to physical function and duration of each of the six stages.

Observations of sweetpotato plantings at Beltsville, Md., in 1955 and 1956, revealed relatively low percentages of natural spread of feathery mottle virosis. None of the leafhoppers in the same location were found to be vectors of the disease. In 1957 the abutilon whitefly, *Trialeurodes abutilonea* (Hald.), was found living on Indian Mallow, *Abutilon theophrasti* Medic. When an August drouth defoliated the weed, this insect was forced off the weed and onto the sweetpotato. It was subsequently revealed to be a new virus vector (*1*).

Bennett (*2*) was probably the first to report on the tissue relations of a plant virus vector. By means of a camera-lucida drawing the stylet of the sugar beet leafhopper was shown in feeding position for transmission of curly-top virus. Fife and Frampton (*3*) demonstrated how the vector of curly-top utilizes the *p*H gradient for passing its stylet through the "acid" parenchyma into the "alkaline" phloem tissue, where it feeds.

The tobacco whitefly, *Bemisia tabaci* (Genn.), is known as a vector of several viruses in the subtropics. Pollard (*4*) studied its feeding habits in the Egyptian Sudan of Africa, where it spreads the cotton leaf curl virus and also causes other damage to cotton. In his anatomical studies a large majority of the insect stylets were observed penetrating to the depth of the phloem. His camera-lucida drawing showed the stylet in feeding position. It had perforated the epidermis and penetrated between the cells to the depth of the phloem. As pointed out by Avidov (*5*), this adult whitefly is well-qualified as a virus vector in Israel, where it is active about 5 weeks in the summer and 10 weeks in the winter. The same insect, *B. tabaci*, was also reported to be the vector of sweetpotato virus B in East Africa by Sheffield (*6*); subsequently, Girardeau (*7*) found that *B. tabaci* was the vector of a sweetpotato mottle mosaic in Georgia.

Concurrently, it was discovered (*1, 8*) that the whitefly, *Trialeurodes abutilonea* (Hald.), was the natural vector of sweetpotato yellow dwarf (feathery mottle) in Maryland; yellow dwarf is probably the same disease as was reported from Africa, Israel, and Georgia (*6, 7, 9*).

Direct micrurgical examination of the abutilon whitefly preceded the anatomical studies of the fixed material. The adult was the first and only stage of *T. abutilonea* observed in the field at Beltsville in late June each year since 1957. This means that pupae are the overwintering stage.

After the crawlers had been hatched, ordinarily they had stopped moving around and were feeding within an hour. After 2 days the rooted cuttings were ready for planting in peat pots for observation of the subsequent stages of development in the insect's life cycle. During 1958, five to ten larvae were transferred onto each of 50 lots of diseased cuttings and carried through to maturity with a percentage survival of 65. These transfer studies were repeated during 1959 and 1960, with essentially the same results.

A time interval of about 3 days was

noted as the span for each of the six stages (including the egg) of the life cycle of the abutilon whitefly, for a generation time of 18 days; but by mid-September the interval had increased to 4 days, to extend the generation time to 28 days. During the latter half of September, the biological activity of the active stages of this insect changed abruptly. By the end of the month none of the pupae had become adults, practically none of the eggs had hatched, and all activity had ceased.

The generation time and breeding activity of the nonvector greenhouse whitefly, *T. vaporariorum* (Westw.), were practically identical to that of the abutilon whitefly vector during the growing season until toward the end of September when instead of stopping it only slowed down and continued activity until frost.

Microscopic examination revealed that once the first larval or crawler stage comes to rest and inserts its stylet into feeding position, the body ceases to use its legs, which are then shed with the skin during metamorphosis into the second larval stage.

The stylet of the first larval stage of the abutilon whitefly appeared to be of uniform size throughout its length and measured about 1.6 μ in diameter, whereas the adult stylets measured about 2.2 μ in diameter.

According to Hargreaves (*10*), the stylets of all larvae and adults of the greenhouse whitefly consist of two tubes composed of two mandibles and two maxillae. The stylets of some larvae may be withdrawn within the body.

Transmission experiments involved the transfer of three or more adults from the source or location of the pupal cases on the diseased plant to the new feeding site on a healthy plant. Positive transmission was obtained in a few instances—or less than 20 percent of the time—when the viruliferous adults survived a day or longer after transfer.

Anatomical studies were necessitated when the micrurgical method failed to clarify the role of the larval stylet in relation to pathogenesis of sweetpotato tissue. In preparation, the three larval stages of *T. abutilonea* were collected and fixed. Three different fixing fluids were used: (i) Newcomer's (*11*), (ii) formalin-acetic acid-alcohol, and (iii) formalin-propionic acid-alcohol. The third fixing fluid proved best. Before the material was fixed, the sweetpotato leaf tissue was cut into oblong rectangular pieces with the larvae oriented lengthwise to facilitate making cross and longitudinal sections. The tissue was dehydrated in tertiary butyl alcohol, embedded in paraffin (55°C), sectioned about 12 μ thick, and stained with Flemming's triple stain.

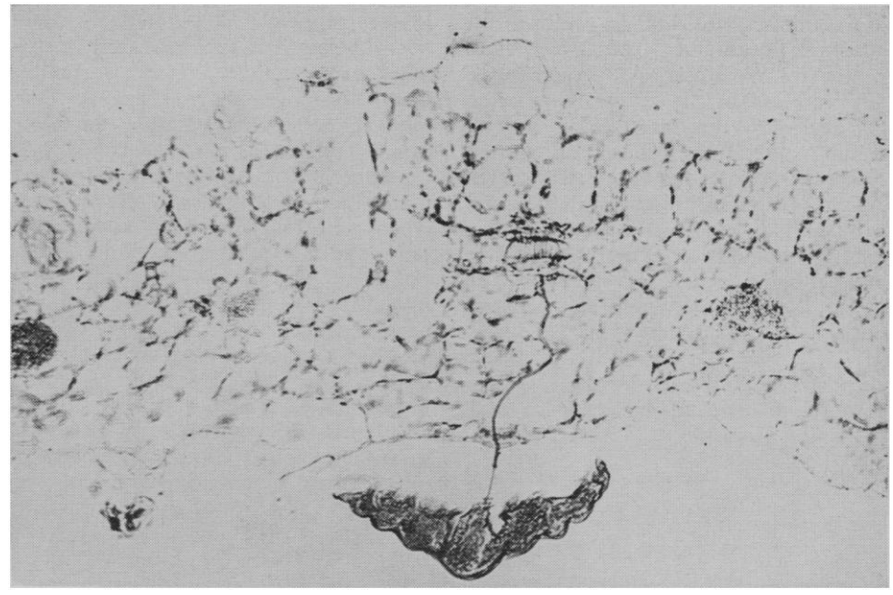

Fig. 1. Cross section of a larva in feeding position on the underside of a sweetpotato leaf. Its sheathed stylet is exposed to view throughout its length by a stained gelatinous secretion. [H. Dermen]

Figure 1 is a microtome cross section which shows an abutilon whitefly second-stage larva in feeding position with its stylet exposed to view throughout its length and ending in a host cell. To observe the full length of a stylet from the rostrum to the depth of the phloem required the examination of several hundred sections before a perfect or complete specimen was found. Examination of numerous histological sections revealed that the larval stylets invariably end in phloem tissue and almost always in the cells of the plant's translocation stream. Fortunately the intercellular channel of the tiny stylet is made conspicuous by the stained gelatinous sheath with which it is clothed.

Figure 2 is another excellent specimen of penetration—a longitudinal section of a third-stage larva in feeding position, with its stylet exposed to view throughout its length. As in Fig. 1, the stylet pathway is made conspicuous by its gelatinous sheath. The section was stained very lightly so as not to obscure the path of the stylet from its origin to its termination.

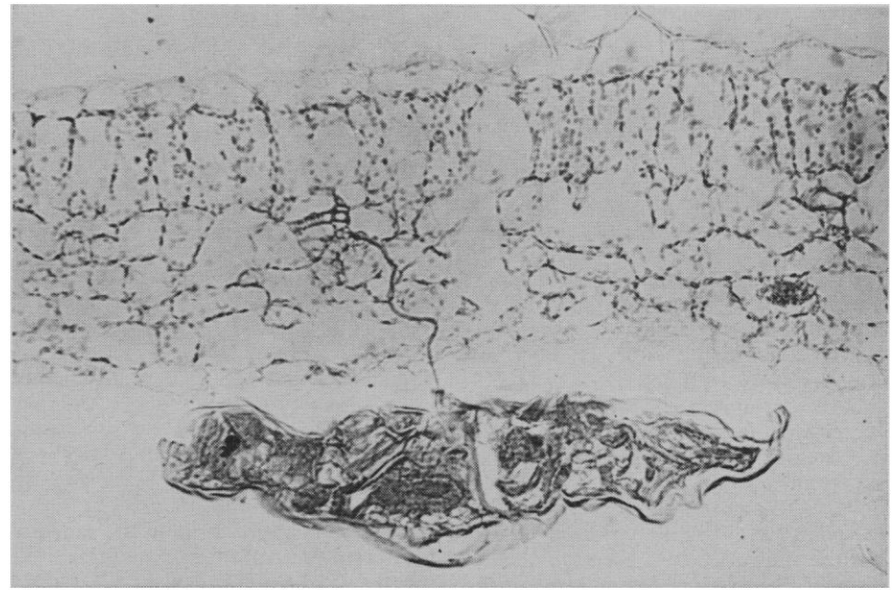

Fig. 2. Longitudinal section of a larva in feeding position with the sheathed stylet completely in view from the rostrum to the stylet's termination at a cell in the plant's translocation stream. [H. Dermen]

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 μ in diameter penetration will be intercellular, because its physical size makes it too limber for direct penetration.

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5 December 1960

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} μg/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 μg 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 *p*H 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 *p*H 6.65 (*1*). When partitioned between ethyl acetate, isoamyl acetate, tertiary amyl alcohol, or peroxide-free diethyl ether and McIlvaine buffers at various *p*H'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 μg 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 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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30 September 1960

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^D and t^A, time periods during which, respectively, reinforcement may be given and reinforcement is never given.

Conventionally, t^D and t^A are held constant and are alternated, and only the first response in t^D 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^D, 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^D time periods, one following