

separated by a constriction from the next section, the proventriculus. In the Gryllidae and Tettigoniidae (e.g., *Conocephalus*, see Fig. 1) the constriction is quite evi-

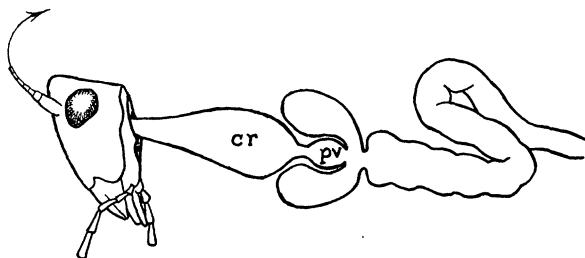


FIG. 1. Head and digestive tract of *Conocephalus fasciatus*, approximately 3 times natural size. The crop (cr) is distended as it is when filled. When the tract is pulled out with the head, it usually breaks at the indentation just posterior to the proventriculus (pv).

dent; in the Acridiidae it is less noticeable. In either case, however, food accumulates in the crop in a state of incomplete mastication. Examination of the crop contents may be most simply carried out by pulling off the head of the freshly killed insect. The digestive tract, at least the anterior part, will remain attached to the head. In crickets, katydids, and meadow grasshoppers the tract usually breaks at the constriction forming the posterior boundary of the proventriculus. In the short-horned grasshoppers practically the entire tract may be pulled out with the head. In either case, the crop may be opened at the anterior or posterior end and its contents pushed out. Teased apart in a drop of water on a microscope slide, the contents are ready for examination. The crop contents of specimens collected years before can be examined successfully if a little more care is observed. The contents of the crop, either from fresh or dried material, if placed under a sealed cover glass in 40 parts of 10% formalin and 60 parts of glycerine, can be studied at leisure.

The value of crop analysis is well illustrated by the fact that studies recently carried out on several species of meadow grasshoppers (Conocephalinae) show conclusively that insects constitute a part of their normal diet. Certain other members of the Tettigoniidae, particularly decticids, are known to be carnivorous (3). The carnivorous habits of the meadow grasshoppers, on the other hand, while known, have been considered aberrant modifications of the normal feeding behavior (1). These first studies, which are to be reported in extended form elsewhere, show further that the vegetative parts of plants are rarely, if at all, eaten by these Conocephalinae. These insects subsist on flowers, pollen, and seeds of grasses, in addition to insects, normally deriving little if any food from the leaves of either the grasses or the broad-leaved plants among which they live. Inasmuch as it has been assumed, largely on the basis of analogy, that meadow grasshoppers exert significant pressure on meadow vegetation (4), the method of crop analysis has in this case demonstrated that the biotic role of this group of insects is quite different from that which has been assumed

for it for many years. These species, which we have believed harmful, may, on the basis of these studies, be considered beneficial, or at least neutral, in their ecological role. An expansion of this technique to the study of the food habits of other species may yield equally interesting and significant results.

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Evidence That Two Different Plant Viruses Can Multiply Simultaneously in the Same Cell¹

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It is well known that a plant may harbor two or more viruses at the same time. Indeed, infection of a plant with two nonrelated viruses, which alone cause only mild symptoms, sometimes results in a severe disease. Thus, mild tobacco mosaic and potato mottle viruses together produce a serious disease of tomato known as streak (?). It has been presumed, but not proved, that two such nonrelated viruses may occupy the same cell. Experiments reported here demonstrate the presence of inclusions of both tobacco mosaic and tobacco etch viruses in a large number of cells of doubly infected tobacco plants. Since these inclusions are closely associated with the respective viruses, their presence together indicates that the viruses increased simultaneously in the same cell.

The type strain of tobacco mosaic virus, which we used, forms abundant hexagonal crystals in the cytoplasm of its host. The mild strain of tobacco etch virus likewise produces crystals, often plate-like, but easily differentiated from those of tobacco mosaic virus, being different in size, form, and location. They occur characteristically in the nuclei but can sometimes be seen in the cytoplasm. Such inclusions were first described for the severe strain of tobacco etch by Kassanis (4).

The crystalline inclusions are usable for identifying tobacco mosaic and tobacco etch viruses within cells. Each of these viruses forms, in the cytoplasm, amorphous bodies which McWhorter (6) has designated as viroplasts. The viroplasts vary in structure; those of tobacco mosaic cannot always be distinguished from those of tobacco etch. They are not, therefore, readily usable for identifying the respective viruses within cells.

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Nicotiana tabacum L. and *N. sylvestris* Spegaz. and Comes were used as host plants for this study. They were inoculated with the two viruses either simultaneously or at intervals of one and four days. Controls consisted of plants inoculated with only one virus. The macroscopic symptoms of tobacco etch usually developed from one to three days before those of tobacco mosaic. Symptoms of both viruses were easily seen in doubly inoculated plants during onset and up to two or three weeks after inoculation. Later, symptoms were predominantly those of etch, doubly infected plants being distinguished from those infected with etch alone merely by a slight reduction in size.

Slides for microscopic examination were prepared by cutting thin slices of tissue, with trichomes attached, from the veins and edges of leaves, and mounting the tissue in 0.85% NaCl. Cytological examinations were made on the 11th and 40th days after inoculation.

The size and location of leaves wherein complete expression of cytological symptoms regularly occurred were determined for both viruses by examination of control plants. Corresponding leaves of doubly infected plants were then studied to determine the distribution of the two viruses in individual cells. The first cytological examination revealed the presence of crystalline inclusions of both viruses in every observed integumentary cell of such leaves from simultaneously inoculated plants. This was not entirely true for corresponding leaves of plants inoculated first with one virus and four days later with the other. In such plants, cells of leaves near the point of inoculation showed inclusions dominantly or entirely of the primary virus only. Cells of leaves higher up the plant, however, nearly always showed inclusions of both the primary and the challenge virus.

When the plants were reexamined 40 days after inoculation, again nearly every observed cell of appropriate leaves showed crystalline inclusions of both viruses. In many cases, leaves near the tops of the plants showed only tobacco etch inclusions while those lower down the plant showed both. This observation suggests that inclusions of etch develop sooner than those of mosaic in old plants and may help explain the dominance of etch symptoms.

The hexagonal plates of tobacco mosaic are known to be closely associated with tobacco mosaic virus. Likewise, the intranuclear crystalline inclusions of tobacco etch are closely associated with tobacco etch virus. Since it is very unlikely that the materials composing these inclusions were formed in distant cells and transferred in toto to the many cells where they were found occurring together, the evidence indicates not only that the two viruses were present in the same cells, but also that they multiplied in the same cells.

Experiments reported above gave no evidence of interference between tobacco mosaic and tobacco etch viruses, such as that reported between the severe strain of tobacco etch virus and potato Y virus (1). The interference phenomenon is well established for bacteriophages (2) and for certain viruses that cause disease in man and other animals (3, 8). It has been demonstrated for plant viruses only in the case cited above. However, the literature on interference occasionally confuses this phenomenon

with *cross protection* such as was observed by Kunkel (5) between tobacco and aucuba mosaic viruses. It seems worth while to point out here that cross protection, in contrast with interference, consists of the protection afforded a plant by virtue of a previous infection with a closely related virus strain. Nonrelated viruses do not ordinarily give such protection.

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Catheterization of the Coronary Sinus, Right Heart, and Other Viscera With A Modified Venous Catheter

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A technique of coronary sinus catheterization (10) has made it possible to study coronary blood flow and myocardial metabolism in intact dogs (6, 7, 11) and has been recently applied to similar studies in man (2, 3).² More recently a modified venous catheter has been devised to meet several of the special problems encountered in this procedure. In extensive trials in man, with Bing and his associates (3), as well as in dogs, the modified catheter described in this report appeared to facilitate catheterization of not only the coronary sinus, but also other regions in the heart and visceral veins.

The venous catheter now most generally used was designed by Courmand and Ranges (4), some years following Forssman's original description of right auricular catheterization in man (8). It is 100 cm long, of the uterular type, with a woven shellacked nylon core covered with a heavy X-ray-opaque plastic coating and with a

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² The application of this technique, together with the nitrous oxide blood-flow method of Kety and Schmidt (12), to the measurement of coronary blood flow in man, has been a joint project undertaken by Bing and his co-workers at Johns Hopkins University Medical School, in cooperation with the authors and with Eckenhoof and his associates at the University of Pennsylvania Medical School (2). To all of these, the authors are deeply grateful for their collaboration and interest, and to Normal C. Jeckel and Edward W. Grant, of the U. S. Catheter and Instrument Corporation, for their help and advice.