Further studies are in progress to determine whether the pituitary principle can be identified with one of the recognized hormonal entities secreted by the pituitary gland.

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X-Ray Diffraction Studies of Inclusion Bodies Found in Plants Infected with Tobacco Mosaic Virus[1]

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Crystalline inclusion bodies in tobacco plants infected with tobacco mosaic virus were observed as early as 1903 (*1–2*), but to date no conclusive evidence has been obtained concerning the nature (or chemical identity) of these bodies. Bernal and Fankuchen (*3*) have shown that purified tobacco mosaic virus exhibits x-ray diffraction patterns arising from the intramolecular arrangement of the atoms within the virus molecule as well as from the intermolecular arrangement of the virus molecules with respect to each other. They showed that the intermolecular spacing varied with the ion concentration and, furthermore, that the order in the gels is two-dimensional but not three-dimensional. Oster and Stanley (*4*) have been able to observe the diffraction of visible light in freshly prepared gels and have calculated a layer spacing of about 3,000 A. More recently, Wilkins, Stokes, Seeds, and Oster (*5*) have reported optical evidence on the layering of inclusion bodies.

We decided to seek further evidence on the growth and development and on the identity and internal structure of the inclusion bodies associated with tobacco mosaic virus, by means of x-ray diffraction studies on inclusion bodies *in vivo*, supplemented by further observations under the microscope. In this paper we report preliminary results.

For the x-ray diffraction studies we used both Norelco and Hilger units, trying copper, iron, chromium, and cobalt radiations. The camera was a North-American Philips microcamera which we had modified by improving the specimen- and film-holders and by increasing the specimen-to-film distance (*6*). We directed the x-ray beam on single, large, rod-shaped inclusion bodies found within hair cells of diseased plants.[2] To date we have obtained a few diagrams from such bodies that show distinct spacings and orientation. The spacings correspond fairly closely to the strongest

[1] This work was made possible by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] The diseased tobacco plants were kindly given us by L. M. Black, of the Brooklyn Botanical Garden.

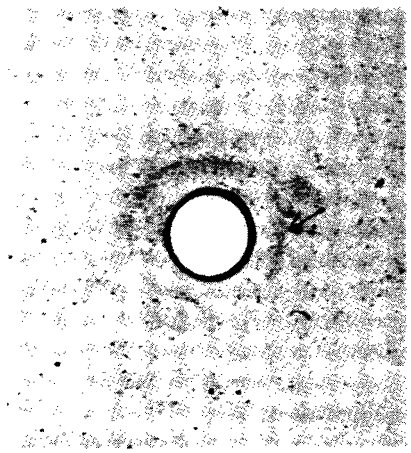

FIG. 1. Enlargement of microcamera diagram of inclusion body, taken with iron radiation. Arrow points to ring of roughly 24 A spacing.

maxima exhibited by the intramolecular diagrams of TMV gels (*3*). They are too few to permit positive identification if the x-ray evidence is taken by itself; in conjunction with the circumstantial evidence reported by others (*4, 5*), indications are strong that the inclusion bodies consist of the virus protein. X-ray diffraction studies on these microscopic objects require highly specialized equipment and techniques. We are still improving both, and intend to report on the instrumental details at a later date. Fig. 1 shows a typical diagram. Microscopic studies are reported by one of us in the note which follows.

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Microscopic Studies of Inclusion Bodies Found in Plants Infected with Tobacco Mosaic Virus[1]

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Inclusion bodies found in the hair cells and leaves of diseased tobacco plants suffering from mosaic dis-

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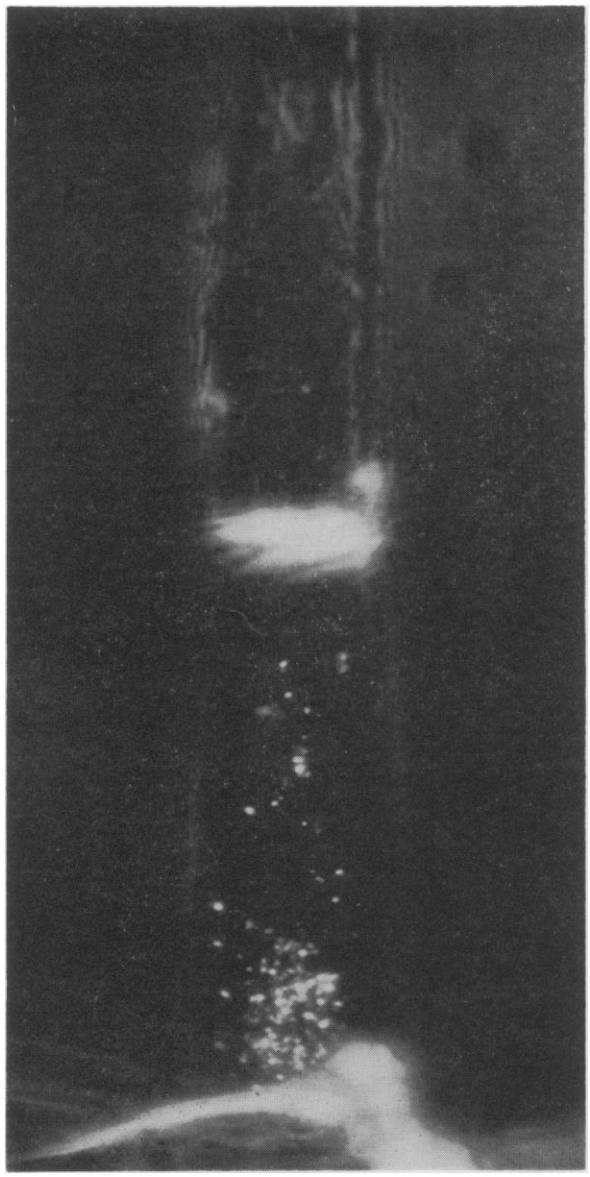

FIG. 1. Microphotograph of hair cell containing scintillating specks. Polarized light with crossed nichols; × 500.

ease have been described by many authors (*1–5*). In connection with our x-ray studies (*6*) this author has made a few observations that are believed to be of general interest.

The inclusion bodies are hexagonal platelets, elongated rods, or aggregates of either form. Generally, the platelets are found in the early stages of the disease. Later platelets, aggregates of platelets, and long rods are met with simultaneously. Still later, the rods predominate.

All these forms are often found to move about within a hair cell, probably under the influence of the flow of the cell juice. They may be stable for long periods, or they may change form within a few hours. Occasionally, 2 inclusion bodies collide. When 2 platelets collide edge-on, they stick together and form a crystalline aggregate with more or less perfect hexagonal edges. On one occasion, the face-on collision of a small platelet with a large one could be observed. For an instant the system appeared to form a round globule, and then a new single hexagon emerged with a larger face than either parent.

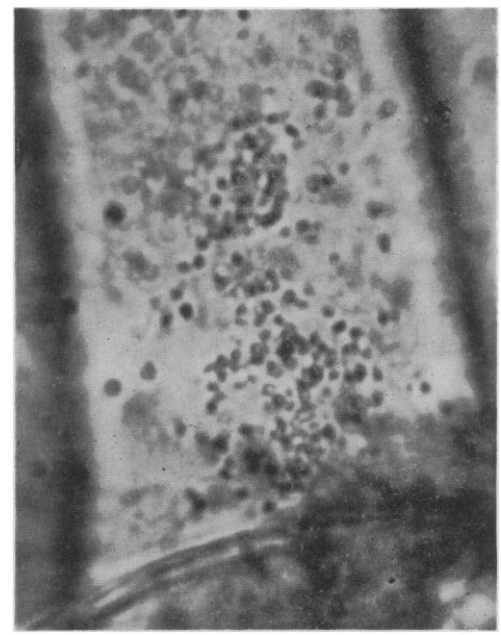

FIG. 2. Hair cell with the same specks. Ordinary light; × 1200.

Under crossed nichols, the long rods exhibit birefringence and so do the platelets when viewed edge-on. Occasionally, lively twinkling against the dark background of the hair cell was observed (Fig. 1). This twinkling is caused by very small, optically anisotropic particles which move rapidly and thereby turn themselves into and out of extinguishing positions. With a magnification of × 1200 or with phase contrast equipment, these particles could be seen without the use of polarized light (Fig. 2).

The scintillating particles were first noticed at a point where a hair was growing a new side branch. Later, they were found in other hair cells on days when no large stable inclusion bodies could be found anywhere in the plant. A large inclusion body that had been selected for mounting in the diffraction camera had disappeared the following day, and no inclusion bodies could be located in those leaves that had been left on the plant. Instead, both in the hair cell that had been positioned in the camera and in the hair cells on the plant, twinkling was observed everywhere. The leaf in the diffraction camera was still in good condition and accordingly was left there another day. The following day, several inclusion bodies were found again in the positioned hair cell, but no scintillating particles were observed. Examination of the plant showed the same over-all picture.

In his paper on the spontaneous formation of struc-

tures in sols, Zocher has described strikingly similar observations (*7*). On standing, V_2O_5 sols tend to form ordered aggregates, and Zocher has also observed twinkling under crossed nichols. This similarity is of special interest because Zocher's ordered colloids belong to a type that has many properties in common with purified TMV solutions.

All in all, the observations reported in this paper are compatible with the hypothesis that the different large inclusion bodies and the small particles responsible for twinkling are forms of aggregation which TMV may assume inside the plant. They suggest that the conditions of stability are different for the different forms.

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Polymorphism of Pregnenolone Acetate

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In this laboratory rigorously purified samples of 5-pregnen-3β-ol-20-one acetate from various sources showed a melting point lower than those reported by previous investigators. This behavior led us to examine the properties of this compound more carefully in order to determine whether polymorphic forms were involved.

Other workers have reported that pregnenolone acetate melts at 146°–147° when prepared from stigmasterol through 3β-acetoxybisnor-5-cholenic acid (*1*), and at 147°–149° when prepared through its semicarbazone after oxidation of cholesteryl acetate dibromide (*2, 3*). In the present work, a sample of pregnenolone prepared through selective reduction of 5,16-pregnadien-3β-ol-20-one from diosgenin (*4*) was acetylated, and the crude product was crystallized from acetone. This was followed by crystallization from isopropanol, ethyl acetate, isopropanol, and acetone. The melting range did not change after the first two crystallizations. When heated from 135° at 0.5°/min, the pure material (prismatic needles, or parallelopipeds, dried *in vacuo* and powdered) partly liquefied at 144.8°–145.5°, solidified at 145.5°–146°, and fused again at 147.5°–150.0°.[1] A sample inserted at 145.5° melted almost completely, then solidified at 146°–147°, and remelted at 147.8°–150.5°. Resolidified samples showed no change below 148°, then melted at 148°–150.5°. On powdering and drying *in vacuo* at 100° or 110° no change in range of fusion was observed, although 1–4% of samples sublimed. Continued high-temperature (145°) treatment caused decomposition as shown by lowered melting points and faulty analyses. The pure material (*Anal.* Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.26; H, 9.65) showed the following specific rotations: $[\alpha]_D^{21} = +18.9° \pm 0.6°$ (1% in EtOH); $[\alpha]_D^{21} = +13.8° \pm 0.6°$ (1% in $CHCl_3$); $[\alpha]_D^{18} = +11.1° \pm 0.6°$ (1% in dioxane).

Further samples of pregnenolone acetate showing the same phenomenon upon melting were prepared from diosgenin (*4*), 3-acetoxy-bisnorcholenic acid (*1*), and cholesterol (*2, 3*). Each sample was recrystallized exhaustively from isopropyl alcohol, acetone, ethyl acetate, and finally benzene-ethyl ether (1:10) to constant melting range. Solubility analyses (*5*) indicated no impurity within the limits of the method. The soluble portions from the solubility analyses and the final recrystallizations were, within instrumental limitations, identical to the main crystal fractions in melting range, optical rotation ($[\alpha]_D^{20} = 10.6° \pm 0.7°$ in dioxane), molecular extinction coefficient at 281 mμ ($\varepsilon = 42.09 \pm 0.31$, 2 mg/cc in absolute ethanol), and infrared absorption spectrum.

The semicarbazone of the purified pregnenolone acetate was prepared in quantitative yield (mp = 253°–255°C dec, inserted at 245°), and recrystallized to constant mp (256.5°–257°C) from chloroform. The pregnenolone acetate prepared by splitting with pyruvic acid (*3*) still exhibited the wide melting range characteristic of polymorphic forms.

On the basis of this physical and chemical evidence, the behavior upon fusion of pregnenolone acetate can be ascribed to the presence of one or more polymorphic forms.

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[1] All melting points are corrected.

Roots, herbs, leaves, and barks still used as healing agents by the contemporary Mayas have been turned over by the Knaggs Expedition to **Sterling-Winthrop Research Institute** for study. Headed by Nelson S. Knaggs, of Hilton-Davis Chemical Company, dye manufacturers of Cincinnati, the expedition visited Guatemala, Yucatan, and Honduras, testing new tropical medicines developed by Sterling-Winthrop, observing Maya methods of weaving and dyeing textiles, and collecting natural-history specimens.

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