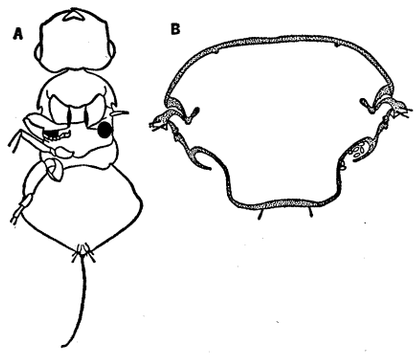

Fig. 2. (A) Ventral side of *Blastophaga jimenezi* (Central American species), with legs in part removed to show position of coxal corbiculae (elongate black spots) and sternal corbiculae (round spots). (B) Cross section of mesothorax of *Blastophaga jimenezi* showing position and shape of sternal corbiculae (pollen grains in place on right side).

without names or mention of function. Grandi (*8*) shows the sternal corbiculae of *Agaon paradoxum* and *Blastophaga estherae*, the coxal corbicula of *Blastophaga aguilari*, and both structures in *Blastophaga williamsi*. Mangabeira (*9*) and Galil and Eisikowitch (*4*) also show the sternal corbiculae of *Blastophaga lopesi* and *Ceratosolen arabicus*, respectively.

Both pairs of corbiculae are present in every species of *Blastophaga* (about 40) I have collected in Venezuela, Panama, Costa Rica, San Andres Island, and Florida, with the exception of two undescribed species, exclusive pollinators of *Ficus tuerckheimii*, which possess only the sternal corbiculae. The African *Elisabethiella stueckenbergi* and the New Caledonian *Blastophaga boschmai* also possess both sternal and coxal corbiculae. Among Old World species, sternal corbiculae only are found in *Blastophaga quadraticeps* (Africa) and *B. jacobsi* (New Guinea), in *Agaon hamiferum modestum* and *Allotriozoon prodigiosum* (Africa), in *Liporrhopalum mindanaensis* (Asia), in *Pleistodontes imperialis* (Australia) and in *Ceratosolen arabicus, C. capensis, C. galili* (Africa), and *C. pilipes* (Borneo) (*10*). Galil and Eisikowitch (*4*) report that as females of *Ceratosolen arabicus* crawl along the narrow exit made by the males among the staminate flowers their bodies become coated with pollen, but they do not mention the presence of any structure to carry pollen in this species.

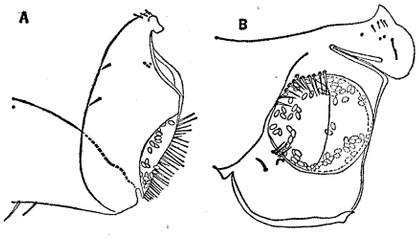

Fig. 3. (A) Coxal corbicula of Venezuelan *Blastophaga* sp. containing pollen grains. (B) Sternal corbicula of Venezuelan *Blastophaga* sp. containing pollen grains.

An unidentified species of Urostigma had an average of 344 seeds per fig in a total of 31 figs examined. A single fig of *Ficus goldmanii* pollinated by one wasp yielded 682 viable seeds. This must mean either that one wasp can pollinate at least 682 flowers (one ovule each), or that apomictic development occurred, or possibly that some pollination leads to apomictic development of other flowers in the same synconium. That the corbiculae can carry sufficient pollen for pollination is revealed by the presence of 404 grains in a single sternal corbiculae of a female *Ceratosolen arabicus*. Usually a fig is pollinated by several wasps, each with two or four corbiculae, assuring an adequate number of pollen grains. There was pollen in the corbiculae of every female wasp emerging from the ripe figs of species of New World Urostigma, but the corbiculae of wasps that had died inside the young figs after accomplishing pollination were empty or contained only few grains of pollen.

Females of *Blastophaga psenes*, the pollinator of the edible fig (*Ficus carica*), lack corbiculae but possess two longitudinal ventral cavities in the head (*11*). Several grains of pollen were present in these cavities in specimens examined, but I cannot determine whether or not these structures serve the same function as the corbiculae because the number of grains of pollen was too small. Much pollen was found, however, in the digestive tract. The ventral side of the head of New World *Blastophaga* females is smooth, not concave.

In the case of 12 species of *Tetrapus* wasps, there were no pollen-carrying structures, but much pollen was found in the digestive tract, as in *Blastophaga psenes*. The numerous anthers in pharmacosyceous figs are located more centrally than the normal and parasitized ovaries (Fig. 1) so that there is no true cavity in the figs. Thus the wasps crawling among the mass of staminate flowers become completely dusted with pollen. The stigmas of the fertile pistillate flowers in the young figs are very long, projecting into the center of the fig, so that it is possible for the egg-laying wasps to scatter pollen on the pistillate flowers while they are laying or searching for pistillate flowers in which to oviposit. Alternatively, *Tetrapus* females may carry pollen internally and regurgitate it on the stigmas of the flowers.

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10. I am indebted to Dr. J. T. Wiebes of Leiden, Rijksmuseum van Natuurlijke Historie and Dr. J. Galil of Tel-Aviv University for identified Old World specimens.
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12. I thank Drs. C. D. Michener and R. C. Jackson, University of Kansas, for reading the manuscript and suggesting various changes, Dr. A. Wille, Universidad de Costa Rica, for stimulating my interest in this work, and the University of Kansas–Universidad de Oriente Program at Cumaná, Venezuela, for opportunities and facilities there.

22 August 1968; revised 25 November 1968

X-ray and Electron Diffraction of Ocular and Bone Marrow Crystals in Paraproteinemia

Abstract. *Crystals in the cornea, conjunctiva, and bone marrow of a patient with a monoclonal gammopathy were analyzed by x-ray and selective-area electron diffraction. X-ray diffraction rings obtained from the abnormal crystalline deposits closely matched cholesteryl stearate patterns, and electron diffraction findings were suggestive of this lipid.*

Corneal and conjunctival crystals visible with the biomicroscope are rare in man. Childhood and adult cystinosis, crystalline corneal dystrophies, and paraproteinemia are the most common

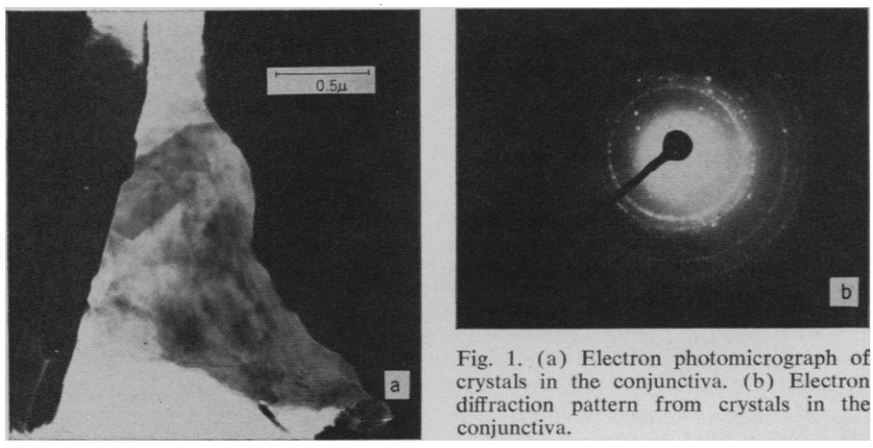

Fig. 1. (a) Electron photomicrograph of crystals in the conjunctiva. (b) Electron diffraction pattern from crystals in the conjunctiva.

diseases in which crystals are found in the eye.

Identification of these crystals has been attempted by x-ray diffraction (*1*) and histological and histochemical examination (*2*). With the use of x-ray diffraction and selected-area electron diffraction, crystals present in the conjunctiva, cornea, and bone marrow of a patient with a paraproteinemia were tentatively identified as cholesteryl stearate. This is the first report of identification of such lipoid crystals in ocular and bone marrow tissue with the use of x-ray and electron diffraction.

The patient, a 45-year-old female, first experienced symptoms in August 1967, when she complained of being dazzled by auto headlights while driving at night. Periods of weakness and repeated nosebleeds were also noted. She was examined in September 1967, at which time vision was 20/25 and 20/20 without glasses in the right and left eyes.

Crystal-like, very fine, countless, polychromatic, iridescent particles were seen in all layers of the cornea and in the conjunctiva of both eyes with the biomicroscope. A complete hematologic and medical work-up was not revealing except for an increase in concentration of gamma globulin in the serum. The sternal marrow had no plasma cells, and Bence Jones protein (characteristic of multiple myeloma) was not found in the urine. Serum lipids and analyses of 24-hour urine samples for amino acids were within normal limits. Repeat examination 5 months later revealed similar findings. At that time, electrophoresis of serum demonstrated an elevated gamma globulin, and immunoelectrophoresis revealed an electrophoretically homogeneous immunoglobulin G (IgG) protein with kappa-type light chains.

Conjunctival and corneal biopsies and sternal marrow aspirate were studied by selected-area electron diffraction and x-ray diffraction for identification of the crystals which, in similar cases, have remained unrecognized.

Very small samples of bone marrow were sandwiched between two electron microscope grids and examined in the JEM-7 electron microscope. Selected-area electron diffraction was used to obtain both single and polycrystalline diffraction patterns. Thin sections of conjunctiva were taken from the patient, fixed in 95 percent formalin or 100 percent ethyl alcohol and examined in the electron microscope; selected-area diffraction studies were made of large crystals observed in the samples. Similar sections from normal conjunctiva were examined as controls and were negative.

Samples of cholesteryl stearate were prepared from a suspension of cholesteryl stearate in distilled water. The suspension was allowed to dry on a collodion-covered grid and then examined in the electron microscope. Electron diffraction patterns obtained for the cholesteryl stearate were compared with those obtained for bone marrow, cornea, and conjunctiva. Normal bone marrow, although not examined in this study, has been reported to yield no x-ray line patterns, with the exception of the hydroxyapatite lines (*1*). The hydroxyapatite lines were not observed in the samples taken from the patient.

Samples of aspirate of bone marrow and conjunctival tissue were also subjected to x-ray diffraction. Bone marrow dried in air was pulverized with an agate mortar and pestle, and divided into two portions. One sample was transferred directly to the outer surface of a fine glass fiber; a very thin coating of vaseline on the fiber was used to cause the powdered marrow to adhere. The second sample was dried with ether and similarly mounted. This sample gave significantly less intense diffraction lines, suggesting that the crystalline material present in the sample was soluble in ether.

Samples of conjunctiva were taken from the patient's eye on two separate occasions. A small section was cut from formalin-treated conjunctiva, rinsed in distilled water, dried, and transferred to the end of a glass fiber for x-ray dif-

Table 1. Comparison of observed x-ray and electron diffraction data with ASTM standard of cholesteryl stearate. The intensities (visual estimates) are rated on a scale of 100 (ASTM); w, weak; s, strong; v, very; and m, moderate.

Electron diffraction			X-ray diffraction				ASTM standard	
			Bone marrow		Conjunctiva		Cholesteryl stearate	
Conjunctiva d(Å)	Bone marrow d(Å)	Cholesteryl stearate d(Å)	d(Å)	I/I_0*	d(Å)	I/I_0*	d(Å)	I/I_0*
		6.07	6.10	m s (50)	5.90	w	6.08	100
		5.55	5.60	v w (10)		v v w	5.57	40
							5.33	10
		4.90	4.99	v w (10)		v v w	4.97	50
4.75	4.77	4.70	4.65	v v w	4.66	v v w	4.65	60
		4.45	4.48	v v w		v v w	4.48	10
4.10	4.16	4.16	4.13	v s (100)	4.10	w	4.15	100
3.60	3.65	3.70	3.72	s (75)	3.70	w	3.80	80
			3.50	v v w	3.47	v v w	3.54	5
							3.24	5
			3.12	v v w	3.10	v v w	3.11	5
2.80	2.87	2.95	2.99	v v w	3.04	v v w	3.02	5
		2.50	2.49	v v w		v v w	2.49	5
							2.40	5
		2.35					2.33	5
2.10	2.27	2.23	2.22	v w (10)	2.23	v v w	2.25	20
1.80	1.82	1.89	1.87	v v w	1.88	v v w	1.89	5

* Visual estimates.

fraction analysis. X-ray diffraction patterns were recorded with a Debye-Scherrer type powder camera (114.59 mm diameter) and V-filtered Cr radiation. Exposure times from 24 to 36 hours were used at 30 kv and 20 ma. The diffraction lines obtained on the photogram were measured and the *d*-spacings and line intensities compared to the available data from the ASTM (American Society for Testing and Materials) card index of standards. Since a very close match to the compound cholesteryl stearate was obtained, a standard sample of commercially available cholesteryl stearate was prepared, and the x-ray and electron diffraction data were compared to the patterns obtained from the bone marrow and conjunctiva samples.

Crystals in the conjunctival sections exhibited a distinct morphology in electron microscope images (Fig. 1a). Similar crystals were also observed in corneal and bone marrow samples. Electron diffraction ring patterns obtained by selected-area studies of crystals in the conjunctival sections are shown in Fig. 1b. Similar ring patterns were obtained for both the corneal and bone marrow samples. The *d*-spacings obtained from the diffraction patterns for the conjunctival and bone marrow samples (Table 1) matched the *d*-spacings for a standard cholesteryl stearate pattern within 5 percent. This was within the allowable limits of accuracy of the electron diffraction method and suggests that the crystals observed in the electron micrographs were cholesteryl stearate.

Missing reflections or incomplete rings in the electron diffraction patterns are quite common, especially when the crystalline material develops a layered or textured structure (Fig. 1a). Very little information is available on the structure of cholesteryl stearate, so that indexing of its lines and electron diffraction intensities was not possible at this stage.

X-ray diffraction Debye-Scherrer data for the bone marrow and conjunctiva have also been recorded in Table 1. The *d*-spacings and intensities were found to match very closely the values of the compound cholesteryl stearate tabulated in the ASTM standards. The *d*-spacings for cystine were also checked, because we first thought that the crystals might be cystine. These values did not compare well with the values obtained for tissue or bone marrow samples. The cause of the crystalline deposition of cholesteryl stearate in a patient with a paraproteinemia is unknown, but the nature of the crystals should provide another clue toward the identification of this disease process.

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5 November 1968

Circadian Clock Action Spectrum in a Photoperiodic Moth

Abstract. *A circadian rhythm of egg hatching in the moth* Pectinophora gossypiella *can be initiated with a brief light pulse. The action spectrum for this effect has a peak in the blue and near-ultraviolet region of the spectrum with a sharp cutoff above 500 nanometers and a more gradual cutoff below 390 nanometers.*

The moth *Pectinophora gossypiella* exhibits a circadian rhythm of egg hatching that can be induced by a single short light signal given after the midpoint of embryogenesis (*1*). This moth also undergoes a photoperiodically inducible diapause, and it is one of the few insects in which photoperiodic time measurement and the biological clock have been studied (*2*). We now report an action spectrum for the light which is effective in initiating the hatching rhythm. Ultimately, it should be of interest to compare this with the action spectrum for photoperiodic induction.

The hatching rhythm persists in continuous red light, but continuous white light damps the rhythm unless the intensity is very low. No rhythm is observed if the developing eggs are maintained at 20°C in darkness after the 5th day of development, regardless of light conditions up to that day, but a rhythm can be initiated with a relatively weak light signal, provided that the signal is given at least 132 hours after oviposition. An action spectrum for the clock controlling this rhythm can be obtained by finding the minimum energy required at different wavelengths to initiate a rhythm. In practice this amounts to finding a threshold value which separates light signals that are too weak from those of saturating effectiveness. The hatching rhythm is restricted to two, or at most three, cycles; and the possible hatching times are spread out over a considerable fraction of each cycle, resulting in a rhythm which is not very sharply defined. However, the light signal can be given at a time when the insects are relatively transparent, still immobile, photoperiodically inducible, and easily handled in large numbers in a small area which can be illuminated with the monochromatic light.

Techniques of culturing the moth and of monitoring the clock-controlled hatching rhythm have been described (*1*). Eggs from adult moths maintained on a light-dark cycle are collected from one night's oviposition. They are then kept at 20°C in the dark; hatching begins in about 10 days and continues for about 70 hours. The number of eggs hatching per hour is monitored by a special fraction collector (*1*). On the 8th or 9th day after oviposition, a rhythm is initiated by a brief light pulse (Fig. 1) always given at the same clock hour.

Light intensity was measured by means of a Yellow Springs Instrument radiometer and a Photovolt photometer model 514. Energies above 1000 erg cm^{-2} sec^{-1} were measured directly with the radiometer. To measure lower intensities the photometer was used in combination with neutral density filters, and the energies were calculated by extrapolation from the radiometer readings. A Carey recording spectrophotometer was used to measure the actual transmission of the neutral density filters.

A slide containing eggs was transferred from the dark rearing box to the exposure box and exposed to light of a particular wavelength and intensity for intervals of 7 seconds to several minutes. It was then transferred in the

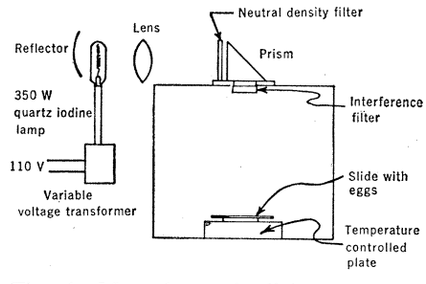

Fig. 1. Monochromatic light source. A partially collimated light beam is projected through a 1-inch (2.54-cm) diameter interference filter onto the specimen. Oriel Optic filters with total bandwidth of 100 Å at one-half peak transmission were used.