BIOCHEMICAL CHARACTERIZATION OF CRYSTALS FROM THE DERMAL IRIDOPHORES OF A CHAMELEON *ANOLIS CAROLINENSIS*

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ABSTRACT

The biochemical characteristics of dermal iridophore crystals from *Anolis carolinensis* have been investigated. Iridophores isolated by collagenase-hyaluronidase treatment were sonicated and their contents fractionated through sucrose. Pure iridophore crystals so obtained were examined by chromatography and electron diffraction. They were found to be pure guanine in hydrated crystalline form. The suggestion is made that the subcrystalline structure of this guanine does not play a role in color production by the iridophore.

Iridophores are the iridescent, white, or blue chromatophores occurring in the integuments, eyes, luminescent organs, and on several internal organs of a large number of invertebrates and nonmammalian vertebrates. These cells have attracted the interest of biologists over the years, first because of their brilliant colors, but later because they were found to be unique among chromatophores in producing nonpigmentary color. Their brilliant colors are instead a function of the structural organization of huge numbers of intracellular crystalline inclusions, occupying the bulk of the cell volume.

In this report, we add *Anolis carolinensis* to the catalog of species whose iridophore crystals have been chemically defined, and we offer a simple procedure for isolating pure crystals that may prove useful in other systems, as well. In addition, since iridophore color is known to arise structurally, we have sought to determine whether the level of structural organization responsible for color selection lies at the macroscopic level of crystal arrangement in the cell or at the level of microscopic crystalline structure. Our results confirm the crystalline nature of the cell's guanine deposits, and strongly suggest that their macroscopic array in the cell alone determines the cell's color.

MATERIALS AND METHODS

Anolis carolinensis, obtained from the Carolina Biological Supply Co., Elon College, N. C., were killed by decapitation and pithing. The animals were swabbed with 70% ethanol, then the whole skins were peeled from them and minced into small pieces in enzyme solution (see below) with a clean, sharp razor blade. The skin pieces were incubated for 16–20 h in a 37°C shaking water bath in Difco TC, Tyrode's solution containing 0.15% collagenase (Worthington CLS, 0.15% hyaluronidase (Worthington HSE) (both from Worthington Biochemical Corp., Freehold, N.J.), 2.5% bovine serum albumin (Sigma Fraction V, Sigma Chemical Co., St. Louis, Mo.), and 1% each penicillin-streptomycin and fungizone Grand Island Biological Co., Grand Island, N.Y.). At the end of this period the skin cells were freed from their digested connective tissue matrix by gentle pipetting,

then pelleted for 30 s at 3,000 rpm in a tabletop centrifuge. The supernate was discarded and replaced with fresh Tyrode's solution containing bovine serum albumin (BSA) and penicillin-streptomycin and fungizone (psf) as above. After three such washes, the cleaned pellet was suspended in fresh Tyrode's solution containing BSA and psf, and centrifuged over a step gradient of 1.8 M and 2.5 M sucrose in Tyrode's solution (no other additives) at 2,000 *g* for 20 min. The pellet of cleaned, isolated cells was drained in the cold, and resuspended in Tyrode's solution containing 2% Triton X-100 (Sigma Chemical Co.). The cell suspension was aspirated and left to stand for a few minutes. This and all subsequent operations were conducted at or below 4°C.

The Triton treatment was found to lyse the isolated cells differentially, so that the cell suspension was greatly enriched for iridophores. These were cleaned and collected as a pellet by centrifugation through 2.5 M sucrose in Tyrode's at 2,000 *g* for 20 min. The resulting iridophore pellet was drained and resuspended in Tyrode's containing 1% Triton X-100 and 0.25% ethylenediaminetetraacetic acid (EDTA).

Purification of the Crystals

The cells were sonicated on a Heat Systems Sonifier (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.), at the highest rheostat value at setting no. 2 for 30 s, then centrifuged over a step gradient of 1.7 M and 1.9 M sucrose in Tyrode's solution at 13,000 *g* for 20 min. Nuclei, melanin granules, and large cell fragments went to the bottom of the tube, and the crystals plus some contaminating filamentous material remained in the 1.7 M sucrose step as a visibly white fraction. This crystal fraction was collected and centrifuged through 2.4 M sucrose in Tyrode's at 165,000 *g* for 1.5 h in an SW 50L head on a Beckman Model L-2 ultracentrifuge (Beckman Instrument, Spinco. Div., Palo Alto, Calif.). The pellet was drained, suspended in 0.5 M Tris-HCl buffer, pH 6.8, containing 2% sodium dodecyl sulfate (SDS), 5% mercaptoethanol, and 10% glycerol (sampling buffer for polyacrylamide gel electrophoresis), and heated for 2 min at 100°C. The crystals, unaffected by sampling buffer or heating, were pelleted at 31,700 *g* for 20 min, washed twice with distilled water, and then examined as follows.

CHROMATOGRAPHY: The washed crystals were dissolved in a small volume of 1.0 N HCl and run on chromatograms against authentic guanine, hypoxanthine, xanthine, adenine, and cytosine dissolved the same way. Two solvent systems were used for thin-layer chromatography (19) on cellulose plates (Eastman Kodak Co., Rochester, N. Y.) both separately and sequentially in two dimensions: (*a*) methanol-HCl (sp gr 1.18)-water (70:20:10, vol/vol), and (*b*) *n*-butanol-methanol-water-ammonia (sp gr 0.90) (60:20:20:1, vol/vol). Paper chromatography (23) was carried out on 3 MM paper (Whatman) in isopropanol-HCl (sp gr 1.18)-water (65:16.7:18.3, vol/vol), or in this solvent system followed by a second solvent system run in the same direction—isobutyric acid-ammonia (2.0-2.3 N) (66:34, vol/vol). Spots were detected with UV light.

ELECTRON MICROSCOPY: The washed crystals were pelleted, fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 6.8, containing 8.6% sucrose, washed in the same buffer, postfixed in 1% osmium tetroxide in the same buffer without sucrose, dehydrated through an acetone series, and embedded in Epon-Araldite. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome with a diamond knife, stained 5 min each with 1% uranyl acetate in 70% ethanol and with Reynold's lead citrate (20), and viewed with a Philips 300 electron microscope. A similar preparation of the crystal pellet *before* the sampling buffer wash was also examined.

ELECTRON DIFFRACTION: The washed crystals were suspended in distilled water and a drop of the suspension was placed on a 0.3% Formvar-coated electron microscope grid. After 1 min, the excess liquid was sucked off with lens tissue, and the grid was air dried. It was coated with a thin layer of carbon in a Denton evaporator (Denton Vacuum, Inc., Cherry Hill, N.J.). Similar preparations were made with authentic guanine (Sigma Chemical Co.). Duplicate samples of iridophore crystals and authentic guanine were further dried for 5-6.5 h under 35 mtorr vacuum in a Denton vacuum DFD-2 freeze dryer fitted with a liquid nitrogen cold trap. All specimens were examined at 1,000 kV or 300 kV on a JEM-1000 high-voltage electron microscope, and their electron diffraction patterns were recorded, analyzed, and compared with guanine diffraction patterns recorded in the literature. The microscope was calibrated with a gold standard.

RESULTS

As was shown previously (21), *Anolis* skin cells can be freed from their connective tissue matrix by collagenase-hyaluronidase treatment. Such enzyme treatment for a period of 12-14 h yields viable cells of all types: epithelial cells, fibroblasts, melanophores, xanthopores, erythrophores, and iridophores. These cells can be maintained in culture for several days (unpublished observation). The prolonged enzyme treatment used in this study, however, tends gradually to destroy all cell types except iridophores, which are particularly refractory to a number of harsh treatments (see below), probably because of their highly reinforced ultrastructure (21). Cell preparations were enriched in iridophores by enzyme treatment of 16 h or more. This was followed by centrifugation of the washed cells through 2.5 M sucrose to remove keratin, and by further treating the cell pellets with 2% Triton X-100 in the cold. This treatment, to which, again, iridophores were more refractory than the other cell types, when combined with a

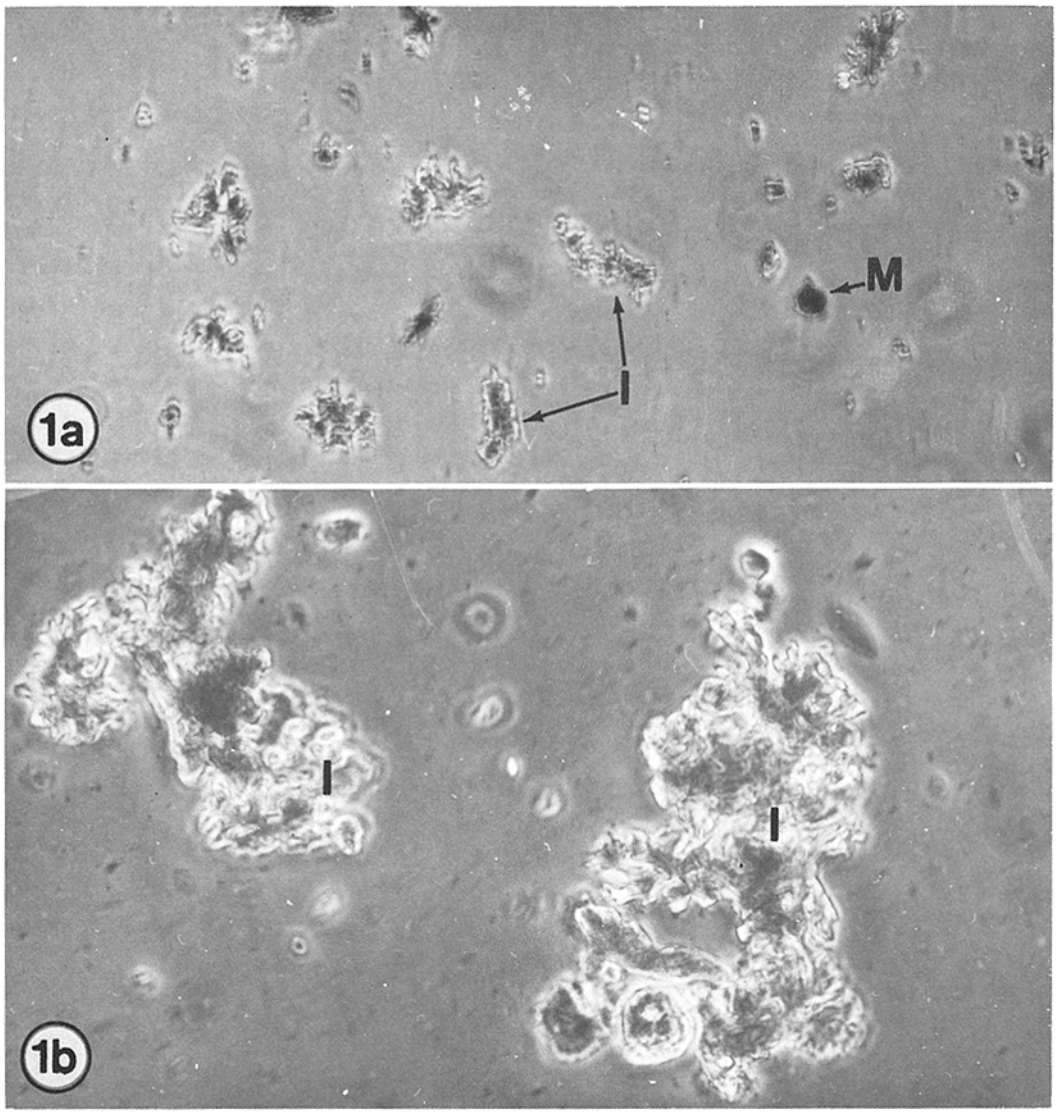

FIGURE 1 Light micrographs of cells from *Anolis* skin, enriched for iridophores by 2% Triton X-100 treatment (see text). Fig. 1 *a* shows many iridophores, *I*, and a single rounded-up melanophore, *M*. Fig. 1 *b* is an enlarged view of two iridophores, showing their morphologically healthy appearance. 1 *a*, × 130; 1 *b*, × 3,280.

further process of again collecting whole cells by centrifugation through sucrose, produced a highly enriched iridophore pellet (Fig. 1 *a*). By light microscopy, the cells appeared unfragmented and morphologically normal, as shown in the higher magnification view (Fig. 1 *b*), and in reflected light they still yielded their typical blue-green iridescence.

If such iridophores were homogenized with a Teflon pestle homogenizer or with a Polytron homogenizer at setting no. 10, osmotically shocked with distilled water, frozen and thawed several times, or suspended in up to 3% Triton X-100, they were not broken up at all or were disrupted only very slowly and incompletely. The only method of cytolysis found to be quickly and completely effective was sonication for 30 s. Fractionation of the Triton X-100 and EDTA-sonicated cells through sucrose step gradients yielded a pellet with only two components: crystals, lacking their

bounding membranes, and filamentous debris. This preparation is depicted in Fig. 2.

The crystals were cleaned of all associated material by suspension of the crystal pellet in SDS gel sampling buffer and heating to 100°C for 2 min. The crystals themselves were unaffected by this treatment, and could be washed and spun down into a pure pellet as depicted in Fig. 3. This pure, cleaned crystal preparation was characterized in a number of ways.

Chromatography of iridophore crystals dissolved in 1.0 N HCl against standards similarly dissolved showed that the crystals were pure guanine. In single-dimensional thin-layer and paper chromatography, the crystals always ran with the authentic guanine and differently from cytosine, adenine, hypoxanthine, and xanthine in three different solvent systems (see Table I and Fig. 4 *a*, *b*, and *d*). A two-dimensional thin-layer chromatogram, using two different solvent systems

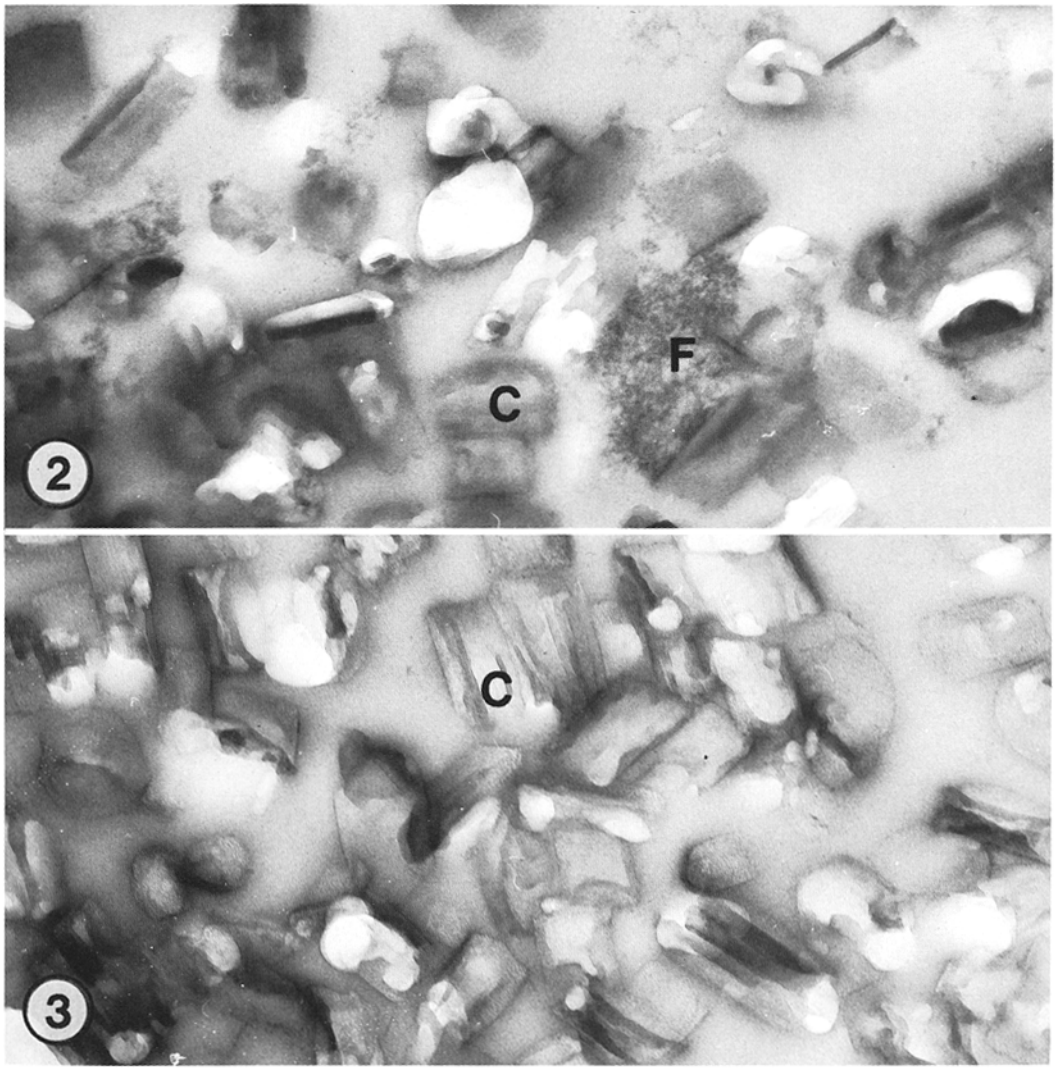

FIGURE 2 Thin section from crystal pellet after sonication in the presence of Triton X-100 and EDTA. Note the intact crystals, *C*, and filamentous material, *F*. × 85,750.

FIGURE 3 Thin section from a preparation identical to that shown in Fig. 2, but after extraction with SDS gel sampling buffer at 100°C for 2 min and two water washes. Note that the filamentous debris is gone, but the crystals, *C*, remain intact. × 85,750.

TABLE I

Chromatography of Isolated Iridophore Crystals and Base Standards

Specimen	Solvent no. 1*		Solvent no. 2‡		Solvent no. 3§	
	Exptl R_f‖	Reported R_f¶	Exptl R_f‖	Reported R_f¶	Exptl R_f**	Reported R_f‡‡
Cytosine	0.44	0.48	0.43	0.43	0.46	0.51
Adenine	0.27	0.30	0.49	0.49	0.35	0.40
Guanine	0.15	0.20	0.27	0.25	0.22	0.29
Hypoxanthine	0.25	0.31	0.36	0.34	0.31	0.28
Xanthine	0.25	0.26	0.21	0.21	0.24	0.25
Iridophore crystals	0.14	—	0.26	—	0.22	—

* Methanol-HCl (sp gr 1.18)-water (70:20:10, vol/vol).

‡ *n*-Butanol-methanol-water-ammonia (sp gr 0.90) (60:20:20:1, vol/vol).

§ Isopropanol-HCl (sp gr 1.18)-water (65:16.7:18.3, vol/vol).

‖ Thin-layer chromatography on cellulose sheets (Eastman Kodak Co.).

¶ Randerath, K., and E. Randerath. 1967. Thin-layer separation methods for nucleic acid derivatives, *In* Methods in Enzymology. Vol. XII A. L. Grossman and K. Moldave, editors. Academic Press, Inc., New York. 330–332.

** Paper chromatography on 3 MM chromatography paper (Whatman).

‡‡ Schwarz/Mann Radiochemical Catalog. 1970/71. Values for paper chromatography on Whatman 40 paper. 51.

sequentially, resolved only a single spot when the crystal sample and authentic guanine were mixed and spotted together at the origin (Fig. 4 *c*). A one-dimensional paper chromatogram, developed in two solvents sequentially, again showed a single spot for the authentic guanine-iridophore crystal mixture. This spot ran with guanine spotted separately, and differently from the other standards (Fig. 4 *e*).

High voltage electron diffraction (with 1,000 kV) of the iridophore crystals and of the guanine standard confirmed an identity between these two substances, and further revealed that the guanine in *Anolis* iridophores is present in crystalline rather than amorphous form. Fig. 5 *a* and *c* show typical patterns obtained from iridophore crystals and authentic guanine crystals, respectively. These specimens have been air dried onto the grid from a water suspension. Fig. 5 *b* and *d* show the corresponding selected areas, that is, the transmissive mode images of the crystals giving rise to the diffraction patterns. The round area is defined by a 10-μm selected area aperture, which limits the diffraction field to a diameter of 2,500 Å, so that diffraction patterns from single small crystals may be obtained. The patterns depicted in Fig. 5 *a* and *c* are almost identical. The reason that the pattern in Fig. 5 *c* appears double, with the duplicate rotated by 90°, is that two crystals lie within the selected area, with their long axes nearly perpendicular to each another. Table II lists the Bragg lattice spacings for two air-dried samples each of iridophore crystals and authentic guanine crystals. The values are obtained from the Bragg equation and the measured spot positions in the diffraction patterns.[1] As is clear from this Table, the most intense spots, namely, those corresponding to Bragg spacings of 3.82 Å and 3.84 Å, are present in the patterns from both the authentic guanine and the iridophore crystals. Several of the Bragg values for less intense spots are also identical or similar for both specimens.

The values for anhydrous guanine reported by Barraud et al. (5) are given in Table III. Clearly, the most intense spot in all of their samples, giving a Bragg spacing of 3.2 Å, is not present in any of the patterns from the air-dried specimens. It is also absent from the diffraction patterns obtained from

[1] Lattice spacings, d, were calculated from the Bragg equation: $d = (n\lambda)/(2 \sin\theta)$, where n is the order of diffraction, in this case 1, λ is the wavelength used, in this case 0.0087 Å for electrons accelerated through 1,000 kV, and θ is half the angle between the transmitted beam and a given beam scattered by the crystals. Since the recording film is far from the specimen, θ is small and $\sin\theta \simeq \tan\theta \simeq \theta$. This makes possible the simplified expression (12): $d = (2n\lambda S)/D$, where d, n, and λ are as above, S is the distance between specimen and film, 1.015 m in this case, and D is the measured diameter of the diffraction rings (or the distance between spots that are 180° removed from one another on the pattern and equidistant from the central bright spot). D was measured on the film negatives with a Bausch and Lomb measuring magnifier.

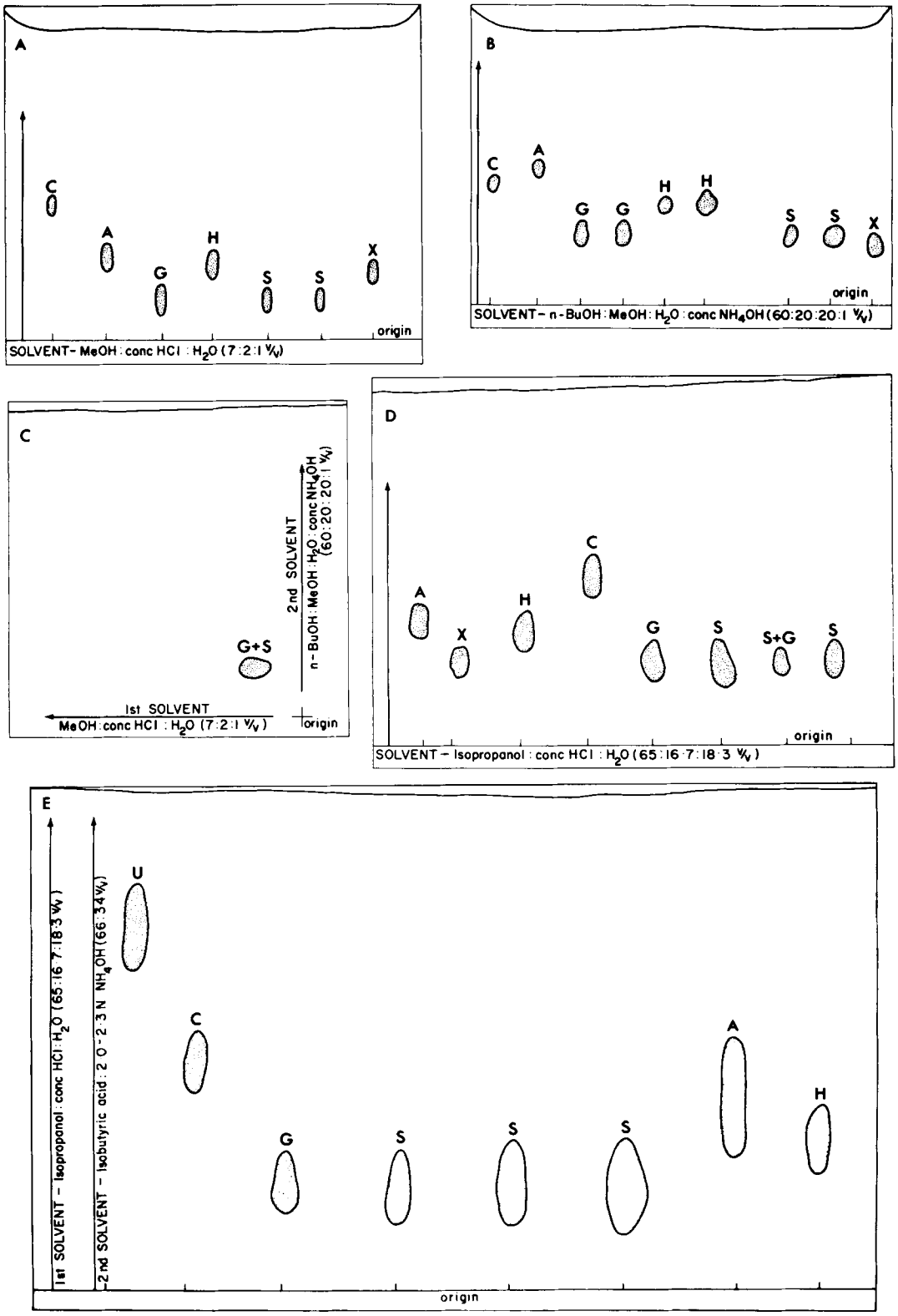

FIGURE 4 Thin-layer (Fig. 4 *a–c*) and paper (Fig. 4 *d,e*) chromatography of purified iridophore crystal preparations, *S*, dissolved in 1.0 N HCl, and similarly dissolved authentic standards: guanine, *G*, hypoxanthine, *H*, xanthine, *X*, cytosine, *C*, adenine, *A*, and uracil, *U*. Arrows show direction of chromatogram development; developing solvents are marked on the figures. UV light was used to detect the spots.

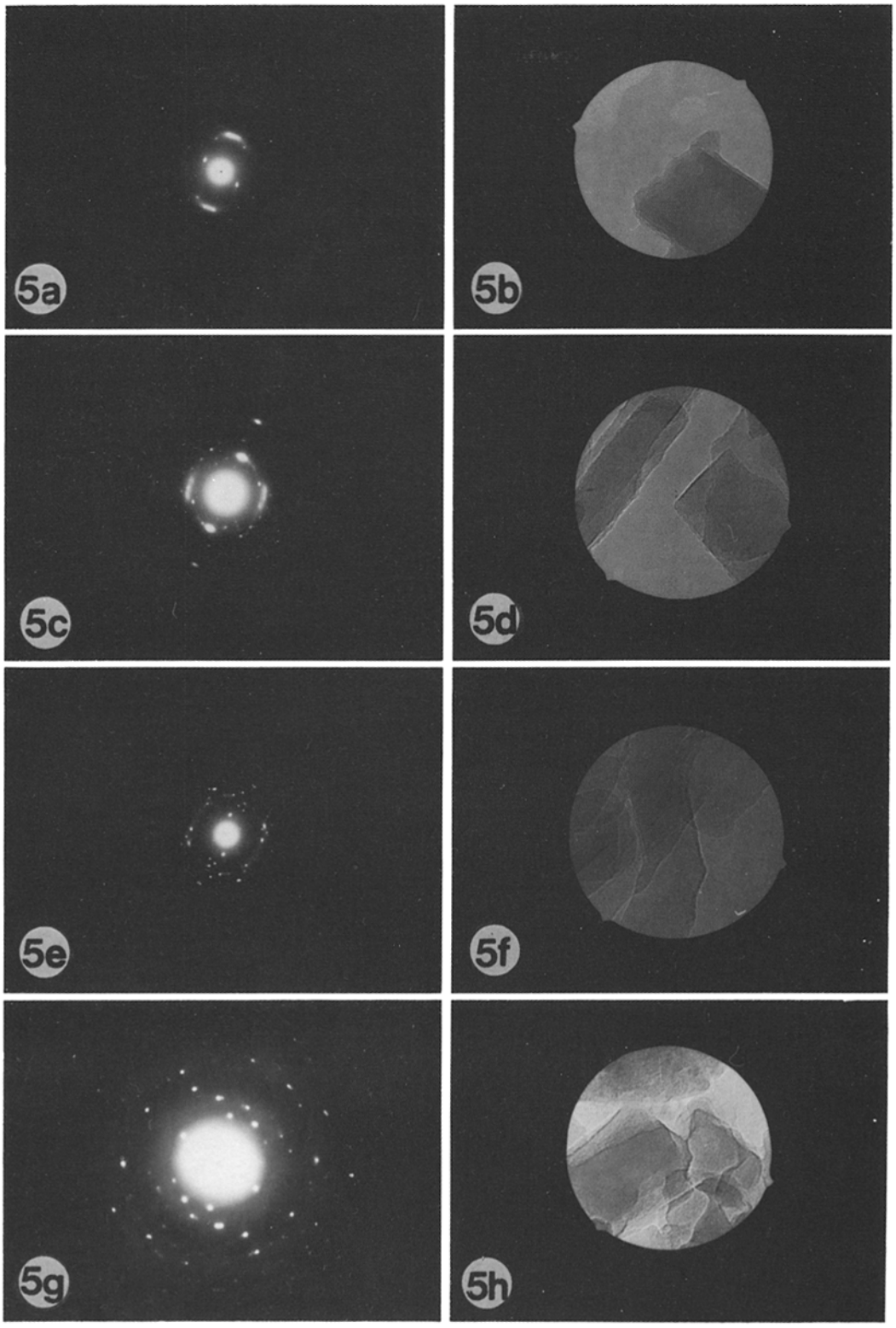

FIGURE 5 High voltage (1,000 kV) electron diffraction patterns and corresponding selected area transmission images of iridophore crystals and guanine standards. Fig. 5 *a*, *b*: air-dried iridophore crystals. Fig. 5 *c*, *d*: air-dried guanine standard. Fig. 5 *e*, *f*: vacuum-dried iridophore crystals. Fig. 5 *g*, *h*: vacuum-dried guanine standard. Fig. 5 a, c, e, g, × 2.5 Fig. 5 *b*, *d*, *f*, *h*, × 112,500.

TABLE II

Diffraction Patterns from Iridophore Crystals and Guanine Standards

Guanine I (air dried)‡	Guanine II (air dried)‡	Iridophore crystals I (air dried)‡	Iridophore crystals II (air dried)‡)
Å*	Å*	Å*	Å*
10.70			10.50
9.82	9.82		
	7.36		
		7.21	
	7.07		7.07
			6.94
			5.65
5.43			5.40
4.92			
			4.59
4.37	4.42		
	4.31	4.31	
	4.26	4.26	
	4.16		
	4.02		
3.89			
3.84		*3.84*	3.84
3.82	*3.82*	3.82	*3.82*
			3.76
			3.70
	3.43		
3.27	3.30		
			2.92
2.72			
			2.64
2.0			
1.94	1.90	1.92	*1.91*

Lattice spacings (Å) calculated from electron diffraction patterns. Values from intense spots are in italics.
*Calculated from the Bragg equation and measured spot positions on the diffraction pattern, as explained in Results.
‡ Two guanine standard samples and two isolated iridophore crystal samples suspended in water, dropped on a 0.3% Formvar-coated grid, drained after 1 min, air dried, and carbon coated. Electron diffraction patterns obtained on a JEM-1000 high-voltage electron microscope, 1,000 kV. Representative diffraction patterns shown in Fig. 6 *a* (iridophore crystals, air dried, I) and Fig. 6 *c* (guanine, air dried, I).

the same specimens at 300 kV. These lower voltage patterns yield Bragg spacings identical to those obtained from high voltage (1,000 kV) patterns.

However, air-dried specimens of authentic guanine and iridophore crystals further dried for 5–6.5 h under vacuum in the presence of a liquid nitrogen cold trap do give patterns essentially identical to those reported for anhydrous guanine. Sample patterns from the standard and iridophore crystals are shown in Fig. 5 *e* and *g*, and the corresponding selected area transmission images in Fig. 5 *f* and *h*. The calculated Bragg spacings are listed in Table IV. They correspond well with the literature values for anhydrous guanine. In particular, both samples give intense diffraction spots corresponding to a Bragg spacing of 3.2 Å.

It is therefore suggested that the electron diffraction patterns recorded from air-dried specimens are patterns from hydrated guanine. Without more information than is available from the electron diffraction patterns shown here, the crystal lattice spacings of hydrated guanine cannot be

TABLE III

Diffraction Patterns from Iridophore Crystals and Guanine Standards

Guanine (A.S.T.M)*	Guanine (Standard)*	Iridophore Crystals*
Å	Å	Å
	9.05	9.03
		8.51
	8.25	
7.90	7.81	
		6.71
6.32 (*70*)	*6.29* (*33*)	6.29
5.01	4.98	
	4.83	4.83
	4.72	
4.62	4.59	
		4.51
4.30	4.30	
	4.19	4.19
4.07	4.06	4.05
	3.90	3.89
	3.75	3.76
	3.62	3.61
3.53 (*50*)	3.52	3.52
	3.46	
		3.40
3.37	3.36	3.35
3.22 (*100*)	*3.19* (*100*)	*3.20* (*100*)
	3.14	3.14
		3.06
3.02	3.01	3.00
		2.96
2.90	2.88	2.90
2.81	2.83	2.83

Lattice spacings (Å) calculated from X-ray diffraction patterns. Values from intense spots are in italics, followed by relative intensities in parentheses.
* Values obtained from anhydrous powder preparations taken from Barraud et al., 1959 (5).

TABLE IV

Diffraction Patterns from Iridophore Crystals and Authentic Guanine

Guanine (Vacuum-dried)‡	Iridophore Crystals (Vacuum-dried)‡
*Å**	*Å**
9.82	
9.07	
7.35	
5.70	
5.13	
4.78	
4.20	
4.11	
4.07	
3.84	
3.76	
3.68	
3.64	
3.53	
3.34	
3.18	*3.22*
3.15	
3.05	3.08
	2.81
2.77	
2.70	
2.68	
2.51	2.50
	2.26
	2.18
	2.04
	1.87
	1.62
	1.50
	1.48
	1.43
1.39	1.40
	1.24

* Calculated from the Bragg equation and measured spot positions on the diffraction pattern, as explained in Results. Lattice spacings (Å) calculated from electron diffraction patterns. Values from intense spots are in italics.

‡ One specimen each of authentic guanine and isolated iridophore crystals, suspended in water, dropped on a 0.3% Formvar-coated grid, drained after 1 min, air dried, and carbon coated, then put under 35 mtorr vacuum in the presence of a liquid nitrogen cold trap for 5–6.5 h. Electron diffraction patterns (Fig. 6 *e*, *g*) obtained on a JEM-1000 high-voltage electron microscope, 1,000 kV.

determined precisely. Still, the Bragg spacings, centering around 3.82 Å, give a good indication of the order of magnitude of the real lattice spacings of the isolated crystals.

DISCUSSION

The discovery that iridophore color arose structurally was made in stages. Early workers noted that iridophores contained birefringent bundles of crystalline material arranged in species-specific patterns (4–6, 22, 25, 28). The crystals could not be extracted with lipid solvents, as could carotenoid pigments (1, 7, 10, 16, 17, 28), nor with water or alcohol as could pteridine pigments (1, 10, 16, 17, 24), nor could they be bleached out as melanins could (10). They were extractable in mineral acids and bases (4, 6, 7, 9, 24, 30), and in alum (4, 6, 7), and they reduced potassium ferricyanide to potassium ferrocyanide (6, 7), all characteristics of guanine.

Within the last 30 yr, iridophore crystals from a number of species have been characterized biochemically by chromatography, spectrophotometry, and degradation with specific enzymes. Preparations examined have ranged from whole-skin extracts (2, 5, 8, 9, 11, 13–15, 26, 29, 30) to crystals scraped from the skin or recovered after cytolysis (3, 18, 27). With few exceptions, the iridophore crystals from all organisms studied have been purines, predominantly guanine. Varying proportions of adenine, hypoxanthine, and uric acid have been found along with guanine in some organisms. Only in isolated cases have iridophore crystals been found to have radically different compositions: Pirie (18) and Dartnall et al. (8) found riboflavin crystals in the iridophores of the tapeta lucida of lemur and bush-baby eyes, respectively; Weitzel et al. (29) found accumulations of zinc-cysteine-hydrate in the tapeta lucida of carnivore eyes, but there is no indication in their paper whether these were crystalline accumulations in reflecting cells comparable to tapetal iridophores; Günder (11) found riboflavin and pteridines instead of purines in the integumental iridophores of some amphibians, and riboflavin in addition to purines in the integumental iridophores of some reptiles.

We have purified the crystals from *Anolis* iridophores to homogeneity, and have shown that guanine is their sole ultraviolet-absorbing constituent, on the basis of paper and thin-layer cellulose chromatography in four different solvent systems. This does not preclude the presence of additional material which was not detected by the UV analysis of our chromatograms.

The electron diffraction results confirm that the cells' guanine deposits exist in crystalline form and suggest that the crystals are hydrated to some

degree. This interpretation is based on the following data. The only diffraction patterns obtained from biological guanine in the past were X-ray powder patterns of dehydrated material from teleost luminescent organ iridophores (5). The values for Bragg lattice spacings from these patterns were found to be identical to the values from powder patterns obtained from commercial anhydrous guanine standards and to values reported for anhydrous guanine in the literature. Selected area diffraction done on the high voltage microscope, as reported in this paper, allows selection of a small enough area to obtain diffraction patterns from single small crystals (2,500 Å). The sample and standard observed in this study, simply air dried onto the grid from an aqueous suspension, give internally consistent values for Bragg spacings that differ from the values reported for the anhydrous powder, and probably represent those of a hydrated form.[2] Since the iridophore provides an aqueous environment for its crystalline inclusions, it is reasonable to surmise that the guanine exists in hydrated form in vivo.

When the sample and standard are dried in a vacuum for 5 h in the presence of a liquid nitrogen cold trap, they become anhydrous and give the altered pattern expected from the work of Barraud et al. (5). On the basis of the data we have, we cannot explain the noncorrespondence between the patterns from vacuum-dried iridophore crystals and from guanine standard (Table IV) in the range 1.39 Å–2.51 Å, within the intramolecular range, nor can we rule out the presence of impurities in the iridophore crystals as the possible source of the extra reflections from the iridophore crystals.

In support of our interpretation, however, we point to the major similarities between the diffraction patterns of guanine and the iridophore crystals in Table II, throughout their range, and to the identity of the most intense spots in Table IV. Furthermore, while we cannot definitively exclude the presence of compounds other than guanine in iridophore crystals, our chromatographic data clearly rule out the presence of other purines.

The question of whether or not the crystal substructure contributes to color production in the iridophore can be answered in the negative with some certainty on the basis of the diffraction results. The Bragg spacings for hydrated guanine seem not to have been reported in the literature previously[2] and, because of the difficulty of obtaining large enough crystals of this form for single-crystal X-ray diffraction, its crystal structure has not been determined. Still, the guanine lattice spacings must be of the same order of magnitude as the Bragg spacings reported here. These center around 3.8 Å, which value is three orders of magnitude different from the (blue) wavelengths of interest in iridophore coloration, seeming to rule out any role of subcrystal structure in the interference phenomena producing iridophore colors. The smallest significant level of structure to consider in understanding iridophore color production is, therefore, the smallest dimension of the whole crystal, which is 1,000 Å.

[2] W. M. MacIntyre, personal communication.

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