Green Blood Pigment in Lizards

Abstract. *Three species of arboreal scincid lizards from the southwest Pacific have a green blood pigment which is evident in the whole blood plasma, muscles, and bones.*

In certain arboreal scincid lizards from New Guinea and the Solomon Islands *Sphenomorphus flavipes* (*1*), *Scincella prehensicauda* (*2*), and *Leiolopisma virens anolis* (*3*), the whole blood plasma, muscles, and bones are a striking bluish green to lime green in color. The green color can also be seen in the buccal mucosa when the animal gapes in defensive display, and in the oviparous *virens*, the egg contents appear pale green through the egg shell (*4*). The green pigment of the internal tissues is probably also responsible for the predominantly green external color of both sexes of *virens* and of male *prehensicauda*. In female *prehensicauda* and in both sexes of *flavipes*, the dominant ground color is brownish with darker cross bands (in female *prehensicauda* and one pattern morph of *flavipes*), with a stripe (in a second pattern morph of *flavipes*), or without additional markings (a third pattern morph of *flavipes*). In these lizards a green color is evident only as a faint olive wash in the ground color or more clearly evident in local areas devoid of a darker underlying pigment such as on the snout and in the lateral body blotches of some female *prehensicauda*. In formalin (approximately 10 percent) and ethanol (approximately 70 percent) all green color rapidly disappears and leaves the specimen externally either palid or dark where there is underlying melanin. For this reason museum specimens lack all traces of green pigment.

We assume that the green pigment of the three species is the bilatriene bile pigment biliverdin or a close chemical relative. This is plausible because, of the several mechanisms known to be responsible for green coloration in the internal tissues of animals, only a green bile pigment, biliverdin or a very similar compound, is a mechanism for vertebrates (*5*).

We do not know the exact identity of the green pigment. We have preliminary data for blood samples from four *flavipes*, but these samples were exhausted before an exact determination could be made and there is little prospect of obtaining further live material in the near future. However, the pigment is readily soluble or degradable in formalin and ethanol solutions, and when whole blood is centrifuged, the green pigment remains in the plasma fraction, an indication that the pigment is not bound to the erythrocytes. The color does, however, come out with the precipitate which forms when trichloroacetic acid is added to the plasma, but it is unknown whether the pigment is bound to a macromolecule such as a protein or is simply caught in the descending macromolecular net.

The absorption spectra for two samples of fresh plasma from three *flavipes* (plasma from two individuals were combined in one sample) indicated one maximum at 405 nm, a slightly lower maximum at 662 nm, and a minimum at approximately 510 nm. Plasma from a fourth *flavipes* blood sample which had been refrigerated for several days showed an absorption peak at 413 nm but failed to show a second peak comparable to that in the first sample. The absorption minimum for this "older plasma" was approximately 510 nm. The absorption peaks at 405 and 413 nm are most likely heme porphyrins which are probably not associated with the green color of the plasma. The maximum at 662 nm and the minimum at about 510 nm, however, while not diagnostic, are in the range one would expect with biliverdin or a closely related compound (*6*).

The green blood pigment and the consequent green coloration of certain internal tissues of these skinks is not found in any other amniote, although the condition is approximated by a few anamniotes, most notably in some South American frogs (*5*). Other skinks, even close relatives of the three species, do not seem to have the pigment. During the karylogical survey that revealed the green pigment in the three species discussed here, approximately 37 other species of skinks were examined in the same manner that led to the independent discovery of the pigment in each of the three species. Most of the skinks were from Southeast Asia, the Australian region, and the islands of the southwest Pacific, that is, the same broad zoogeographic area as the known species with the green pigment, and they included one of the closest living relatives of the green-blooded species, *Scincella rabori* from the Philippines. The fact that the green pigment was not discovered in any of these other skinks emphasizes the likelihood that among skinks the pigment is unique to these three species.

Although the most recent generic revision of the subfamily to which these three skinks belong (*7*) places them in three separate and relatively large genera, the apparent uniqueness of their green pigment suggests that they are closely related. Other morphological features plus geographic proximity also imply a close relation. These are the only skinks, for instance, with highly modified, gland-like scales on the ventral tip of the tail, and they are among the few skinks with prehensile tails. These adaptations, plus the less remarkable but noteworthy expanded basal toe lamellae, are indicative of their arboreal life, and in combination make these skinks most distinctive. The three species are also related geographically: all (*virens, flavipes*, and *prehensicauda*) occur on New Guinea, while *virens* extends east into the Solomon Islands.

Prior to the discovery and description of *prehensicauda*, an earlier generic revision (*8*), based primarily on external morphology, indicated that *virens* and *flavipes* were very closely related. Work on the skull osteology of all three species and a reconsideration of their external morphology supported the idea of the close relation of *virens* and *flavipes*, as well as *prehensicauda*, prior to the discovery of the green pigment. With the discovery of the green pigment in the blood, the close relation of the three species is so thoroughly documented as to suggest that they should be placed in a separate genus (*9*).

ALLEN E. GREER
GARY RAIZES
Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

References and Notes

1. W. Hall, personal communication.
2. G. Gorman, personal communication.
3. T. P. Webster, personal communication.
4. F. Parker, personal observation.
5. Teleost fish: A. Willstaedt, *Enzymologia* **9**, 260 (1941); M. Caglar, *Nature* **155**, 670 (1945); *Commun. Fac. Sci. Univ. Ankara* **3**, 265 (1950); D. Fox, *Animal Biochromes and Structural Colors* (University Press, Cambridge, 1953), pp. 278–279; ——— and N. Millott, *Experimentia (Basel)* **10**, 185 (1954). Frogs: A. Barrio, *Physis Rev. Assoc. Argent. Cienc. Natur.* **25**, 137 (1965); C. J. Goin and O. B. Goin, *Copeia* **1968**, 581 (1968). Mammals (dog placenta): R. Lemberg, *Biochem. J.* **28**, 978 (1934).
6. R. Lemberg and J. W. Legge, *Hematin Compounds and Bile Pigments* (Interscience, New York, 1949), pp. 116–117; C. H. Gray, *The Bile Pigments* (Methuen, London, 1953), pp. 33–34.
7. M. Mittleman, *Smithson. Misc. Collect. No. 117* (1952).
8. M. A. Smith, *Rec. Indian Mus. Calcutta* **39**, 224 (1937).

9. A. Greer, in preparation.
10. Supported by the National Science Foundation through grants to E. E. Williams (GB 6944), the Biological Laboratories of Harvard University (evolutionary biology training grants GB 3167 and GB 7346 and undergraduate research participation grant GY 4555), and through a predoctoral fellowship to the senior author. We thank Drs. L. Bogorad, R. Etheridge, E. Mayr, and E. Williams for comments on the manuscript. Dr. W. Brown provided us with living specimens of *Scincella rabori*.

2 June 1969; revised 11 August 1969

Enzyme from Calf Thymus Degrading the RNA Moiety of DNA-RNA Hybrids: Effect on DNA-Dependent RNA Polymerase

Abstract. *An enzyme present in extracts from calf thymus degrades specifically the RNA moiety of DNA-RNA hybrids. Other nucleic acids, such as single- or double-stranded DNA and single- or double-stranded RNA, are not affected to a comparable degree. If prepared free of the hybrid-degrading enzyme, RNA polymerase from calf thymus shows a fivefold increase in activity on denatured DNA as compared to native DNA.*

In experiments on the purification of RNA polymerase from calf thymus, we observed that, in contrast to other reports (*1–3*), the template activity of denatured DNA for a highly purified polymerase preparation is much higher than that of native DNA. However, if less purified enzyme preparations are used, only native and not denatured DNA stimulates the synthesis of RNA. This finding suggested that a factor is present in the raw extract which specifically influences RNA synthesis on denatured DNA. Further studies on this factor indicated that it is an enzyme which specifically degrades the RNA moiety of DNA-RNA hybrids.

The enzyme is contained in a fraction which occurs as a by-product during the course of RNA polymerase purification. Calf thymus tissue was homogenized in 0.01*M* tris, *p*H 7.8, containing 15 m*M* mercaptoethanol. Then $MnCl_2$ and $(NH_4)_2SO_4$ were added to give a final concentration of 2 m*M* and 0.1*M*, respectively. The resulting precipitate consisting mainly of deoxyribonucleoprotein was sedimented by centrifuging at 20,000*g* for 100 minutes. The supernatant was freed from remnants of deoxyribonucleoprotein by filtration through an asbestos filter plate (Seitz, Bad Kreuznach). To the preparation, one-third of its volume of packed DEAE (diethylaminoethyl) cellulose, previously equilibrated with the same buffer, was added. The suspension was stirred at 4°C for 15 minutes and centrifuged (10 minutes, 3000*g*). The supernatant, from which virtually all RNA polymerase activity had been removed, was dialyzed against 0.01*M* tris, *p*H 8, and retained as fraction A for the following experiments. Fraction A can be kept for several weeks at −70°C without detectable loss of activity.

The influence of 0.05 ml of fraction A (25 μg of protein) on the standard polymerase assay is shown in Table 1. The assays contained in a total volume of 0.5 ml: 0.03*M* tris, *p*H 7.8; 1 m*M* $MnCl_2$; 10 m*M* mercaptoethanol; 0.1*M* $(NH_4)_2SO_4$; 0.2 m*M* each of guanosine, cytidine, and adenosine triphosphates (GTP, CTP, and ATP); 0.1 μc of C^{14}-uridine triphosphate (UTP) (50 μc/μmole; Radiochemical Centre, Amersham) and 15 μg of protein of calf thymus RNA polymerase (*4*). Calf thymus DNA (Sigma) and fraction A were added as indicated in Table 1. The mixtures were incubated for 10 minutes at 37°C. After addition of 150 μg of yeast RNA (as carrier) and five drops of a saturated $Na_4P_2O_7$ solution, the acid-insoluble material was precipitated by 4 percent trichloroacetic acid (TCA). The precipitate was sedimented by centrifugation, resuspended in TCA and collected on Millipore filters for counting in a Packard Tri-Carb liquid scintillation spectrometer.

Two effects of fraction A can be distinguished: (i) Incorporation of UTP is stimulated by a factor of 3 if native DNA is used as template. (ii) This incorporation is inhibited by 90 percent if denatured DNA is used. If fraction A is present in the assay, addition of denatured DNA inhibits the RNA synthesis on native DNA. A similar inhibiting effect of denatured DNA on the RNA polymerase reaction has been described by Furth and Ho (*3*).

For further characterization of the active agents, fraction A was chromatographed on a DEAE-cellulose column, and portions of the individual fractions were added to standard polymerase assays containing either native or heat-denatured DNA. The factor stimulating RNA synthesis on native DNA elutes at about 0.03*M* tris, *p*H 7.8, whereas the factor inhibiting RNA synthesis on denatured DNA elutes at about 0.20*M*. Thus, stimulation and inhibition are the effects of two distinct moieties. The purified stimulating factor was not active if denatured DNA was used in the assay, and conversely no effect of the inhibiting factor was observed if native DNA was used.

The nature of the inhibitory effect of fraction A became obvious by the finding that, upon addition of fraction A, the product of the RNA polymerase reaction, when denatured DNA was used as template, was rapidly degraded. By way of contrast, if native DNA was used as template the product remained almost unaffected. It is known from work on bacterial RNA polymerase (*5*) that, if single-stranded DNA is used as template, the reaction product consists largely of DNA-RNA hybrids. Our findings therefore suggested that the factor present in fraction A specifically degrades RNA when it is hybridized to DNA.

In order to test this hypothesis, the single-stranded DNA of the bacterial phage fd (*6*) was used as template for RNA synthesis, and the product was analyzed in a CsCl density gradient. RNA was synthesized under standard assay conditions (see above) with the exception that 7 μg of phage DNA per milliliter and 0.04 μmole of UTP per milliliter (0.2 μc/ml) were used. After the reaction mixture was incubated for 15 minutes, CsCl was added to give a density of 1.71 g/ml. After addition of ^{3}H-labeled phage DNA as marker, the sample was centrifuged for 60 hours at 35,000 rev/min (SW-39 swinging bucket rotor of the Spinco L ultracentrifuge). Fifty fractions were collected. Portions were precipitated with 4 percent TCA together with 150 μg of yeast RNA as carrier, and the acid-insoluble material was collected on Millipore filters and analyzed for radioactivity.

As expected, the ^{14}C-labeled product bands at a density slightly higher than the single-stranded tritiated DNA from phage fd which was added as a marker (*7*). The fractions containing ^{14}C—the yield amounted to more than 90 percent of the total labeled RNA—were collected and dialyzed against a solution containing 0.03*M* tris, *p*H 7.8;