

of newly captured females of this species (20.4 by 11.7 mm) (5). Some eggs from each of the captive animals were incubated artificially, but no embryos developed.

Animal No. 3 escaped from the terrarium on 21 March. It was accidentally stepped on and killed. Autopsy revealed it was nearly ready to lay again. Animal No. 2 died of unknown causes. Autopsy showed its right ovary had almost completely disappeared, and the left ovary contained a few very small ova. This led me to think that perhaps females of this species hatch with a given number of primordial ova in the ovaries. When these have been ovulated, the animal becomes reproductively inactive. This idea was supported by the fact that animal No. 1, after laying a fourth clutch in March, stopped laying. It still retained its breeding colors and appeared perfectly healthy, however.

This hypothesis proved incorrect when No. 1 was autopsied 3 months after it laid its last clutch. It possessed as many eggs in each ovary as No. 3 had at death, although No. 1 laid nearly three times as many eggs as No. 3 (see Table 1). One egg in each ovary was quite enlarged, but neither would have been ready to lay for some time.

At present it is impossible to say how much of this response was due to light and how much to heat. Bartholomew (3, 4) found that light is more important than heat in producing a reproductive reaction in *Xantusia vigilis*. Probably a similar situation exists in this case, since the surface temperatures to which these animals were exposed are only slightly above the minimum required for their normal activity. When these animals burrowed in the sand they reduced their environmental temperatures still further.

They could also reduce the amount of light received simply by burrowing in the sand. However, the environmental temperatures of the cage never went above the maximum these lizards could tolerate. Therefore they were not forced to burrow under the sand by extreme temperatures, as they normally are in their natural surroundings. Consequently, they tended to stay on the surface longer than they usually do in the field, and thus were exposed to light for relatively long periods (6).

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#### References and Notes

1. D. S. Farner, *Ann. Rev. Physiol.* **23**, 71 (1961).
2. G. A. Bartholomew, in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Withrow, Ed. (American Assoc. for the Advancement of Science, Washington, D.C., 1959), p. 669.
3. ———, *Anat. Record* **106**, 49 (1950).
4. ———, *Copeia* **1953**, 45 (1953).
5. Unpublished data in this laboratory.
6. Thanks are due Loree Ostroff for permission to autopsy animals that had been given to her. This research was supported by grant No. G-5480 from the National Science Foundation.

24 July 1961

### Interaction between Riboflavin and Rutin

**Abstract.** Riboflavin-5'-phosphate forms a charge transfer complex with rutin at neutral pH in the same manner as it forms one with tryptophan or serotonin, when the system is observed by quantitative spectrophotometry.

Isenberg and Szent-Györgyi (1) reported that a strong charge transfer complex of riboflavin-5'-phosphate (RFP) can be produced at neutral pH by simple addition of some indoles (for example, tryptophan and serotonin). During studies of the interaction between biologically active natural substances, Nayatani and Yagi (2) observed that rutin, a representative flavonoid pigment, had some inhibitory effect upon photolysis of riboflavin. It is possible that a complex can be formed between rutin and riboflavin and that this complex plays some role in the stabilization mentioned above. We report evidence of interaction between RFP and rutin; we used the

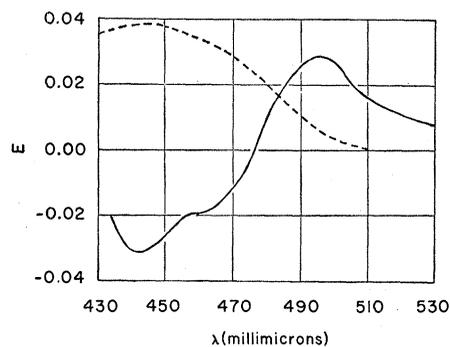


Fig. 1. Absorption curve of RFP-rutin complex in phosphate buffer (pH 7.0) at room temperature (solid line). The RFP concentration is  $5 \times 10^{-5}M$  in both the sample and the reference cell; the rutin concentration in the sample cell is  $2 \times 10^{-4}M$ . The measured extinction is corrected for the rutin absorption. (Dashed line) Absorption curve of a control solution of RFP ( $4 \times 10^{-5}M$ ).

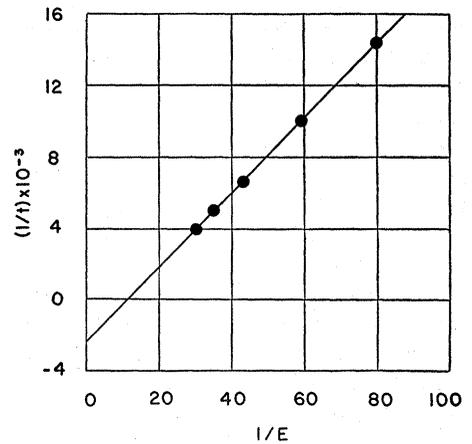


Fig. 2. Plot of the inverse of the rutin concentration ( $1/t$ ) versus the inverse of the extinction of the complex ( $1/E$ ). The RFP concentration is  $5 \times 10^{-5}M$  in both cells.

method of Isenberg and Szent-Györgyi (1).

Upon balancing  $5 \times 10^{-5}M$  RFP with varying concentrations of rutin against  $5 \times 10^{-5}M$  RFP, all in phosphate buffer of pH 7.0, a peak was obtained at  $495 m\mu$ , while a control solution of RFP showed a peak at  $445 m\mu$  (Fig. 1). In the 400- to  $530 m\mu$  region, the measured extinction of the complex was corrected for the considerable absorption of rutin. From the similarity of this shift to the shifts in the spectra of RFP and indoles, we concluded that a strong charge transfer complex is produced, in which a rutin molecule gives one electron to an RFP molecule.

To compare rutin's affinity with the indoles' affinity, the inverse of the dissociation constant of the complex,  $K$ , was obtained by plotting  $1/E$  against  $1/t$ , as shown in Fig. 2. Since the intercept on the ordinate corresponds to  $-K$ , rutin's  $K$  equals 2400 lit./mole;  $K$  for tryptophan equals 60 and  $K$  for serotonin equals 400. It is interesting to note that rutin has much higher affinity for riboflavin than tryptophan or serotonin.

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#### References

1. I. Isenberg and A. Szent-Györgyi, *Proc. Natl. Acad. Sci. U.S.A.* **44**, 857 (1958); ———, *ibid.* **45**, 1229 (1959); I. Isenberg, A. Szent-Györgyi, S. L. Baird, Jr., *ibid.* **46**, 1307 (1960).
2. K. Nayatani and K. Yagi, *Studies (Kobe College)* **4**, No. 3, 43 (1958).

22 September 1961