Biochemistry of Locusts

6. THE OCCURRENCE OF A FLAVIN IN THE EGGS AND OF A PTERIN IN THE EYES OF THE AFRICAN MIGRATORY LOCUST (*LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.) AND THE DESERT LOCUST (*SCHISTOCERCA GREGARIA* FORSK.)

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Previous reports in this series (Goodwin & Srisukh, 1949, 1950, 1951) have been concerned with the major pigment components of locusts; the present paper is concerned with the identification of two minor yellow components, namely, a flavin, in all probability riboflavin, in the eggs and a pterin in the eyes. They occur in both species examined (*Locusta migratoria migratorioides* R. & F. and *Schistocerca gregaria* Forsk.). Riboflavin has previously been reported in *Schistocerca* eggs by Gourevitch (1937) and Busnel & Drilhon (1942) on the basis of fluorescence tests only. It has also been reported in the eggs of another Acridid, *Melanoplus differentialis*, by Bodine & Fitzgerald (1947). Busnel & Drilhon (1942) have stated that pterins occur in *Schistocerca* eyes.

EXPERIMENTAL

Extraction of the flavin from locust eggs. Newly laid eggs or eggs removed from the body cavity of mature females were crushed in a mortar with acid-washed silver sand and ground with small portions of acetone, containing 1% (v/v) of conc. HCl, until no further pigment was extracted. The combined extracts were concentrated *in vacuo* to one-third their original bulk, then diluted with an equal volume of water and extracted with light petroleum (b.p. 40–60°) to remove the β -carotene which is present in eggs in considerable amounts (Goodwin, 1949). The small amount of light petroleum remaining in the pale-yellow aqueous phase was removed *in vacuo* and 0.1 N-NaOH added until the solution was just acid to litmus. The solution which was reddish-brown was chromatographed on acid-washed talc using water to develop the chromatogram. Two zones separated, the upper being greyish and the lower larger zone being yellow; the upper zone was discarded and the yellow pigment eluted with absolute ethanol. This solution was then examined for riboflavin.

Extraction of the pterin from locust eyes. The compound eyes, which were removed from the head simply by making an incision round the eyes and lifting them out with small forceps, were crushed with acid-washed silver sand and extracted a number of times with small portions of water, about 20 ml. being required to extract completely sixty pairs of eyes. The resulting light purplish extract was filtered through a G4 sintered-glass crucible and yielded, on standing, a lemon-yellow slightly turbid solution. This solution was then shaken twice with an equal volume of light petroleum, to remove carotenoids, and then evaporated to dryness *in*

vacuo. The dry solid was extracted with ethanol to remove final traces of lipid material and then dissolved in water to yield a clear lemon-yellow solution. The main pigment was then separated from a small amount of yellow impurity by chromatography on acid-washed talc using water as the developer. The minor yellow component separated from the main pigment and passed rapidly into the filtrate. The pigment was eluted with water, the water removed *in vacuo*, and the properties of the residue recorded in as much detail as possible considering the very minute amounts of material available. A number of batches of eyes of both species was examined in this way, with identical results in every case.

RESULTS AND DISCUSSION

The flavin in locust eggs

The yellow water-soluble pigment obtained from locust eggs was shown to be indistinguishable from riboflavin in a number of ways. Its absorption spectrum (Fig. 1) over the range 550–350 m μ . was

Fig. 1. A comparison of the spectrum of the pigment extracted from locust eggs with that of an authentic specimen of riboflavin. - - - -, Locust pigment; ———, riboflavin.

indistinguishable from that of an authentic specimen of riboflavin (supplied by British Drug Houses Ltd.). At wavelengths below 350 m μ . slight deviations were observed, which were consistent with the

presence of small amounts of absorbing impurities in our pigment; the position of the short-wave band was, however, still identical with that of riboflavin (268 m μ . in 0.1N-NaOH). The pigment was also subjected to the following tests: when dissolved in water or ethanol the pigment exhibits a bright yellow fluorescence in ultraviolet light, whilst in alkaline solution the fluorescence disappears as it does on the addition of sodium dithionite (Na₂S₂O₄), to the aqueous or ethanolic solutions. In the latter case the visible yellow colour also disappears, but on shaking in air both the original yellow colour and fluorescence return. Further, the original fluorescence was not destroyed when an aqueous solution of the pigment was treated with acid potassium permanganate and the excess potassium permanganate destroyed with hydrogen peroxide. All these tests are characteristic of riboflavin.

This demonstration that the flavin present in the newly laid locust eggs of both species is in all probability riboflavin, is interesting, for other investigations are not consistent regarding its occurrence in the newly laid eggs of other insect species. Bodine & Fitzgerald (1947) report that it is present in the newly laid eggs of *Melanoplus differentialis*, but that it disappears during incubation, probably owing to its conversion into pterins. Polonovsky & Busnel (1949), on the other hand, consider the opposite state of affairs obtains in the eggs of *Bombyx mori*; these eggs when newly laid apparently contain pterins, which are converted into riboflavin during embryonic development.

The pterin from the eyes of locusts

The pigment, purified as described above, was easily soluble in water, mineral acids, glacial acetic acid, alkalis and ethanol acidified with hydrochloric acid; it was only very sparingly soluble in absolute ethanol and was insoluble in chloroform and ethyl ether. It had a faintly acid reaction, gave a negative murexide test, gave with ammoniacal silver nitrate a brownish precipitate soluble in dilute nitric acid, and, after boiling in faintly acid solution with a 10% (w/v) copper sulphate solution, gave on standing a yellow precipitate. The original colour was not altered on treatment with zinc and hydrochloric acid. The pigment exhibits an absorption spectrum with the main maximum located at slightly different wavelengths according to the pH of the solvent namely: 288 m μ . in phosphate buffer of pH 7, 292 m μ . in 0.1N-sodium hydroxide, and 278–280 m μ . in glacial acetic acid; a very wide inflexion occurs at longer wavelengths beginning at about 330 m μ ., but only in the phosphate buffer is a slight band ($\lambda_{\max}$. about 345 m μ .) observed. The full curves are recorded in Fig. 2. In ultraviolet light the pigment fluoresces bluish-green when dissolved in distilled water (pH 6.6) or 0.1N-sodium hydroxide

and blue to greenish-blue in glacial acetic acid and dilute mineral acids. The fluorescence is not destroyed by treatment with sodium dithionite, acid potassium permanganate, exposure to ultraviolet light, or heating on a water bath with 10% (v/v) sulphuric acid for 5 min.; it was suppressed at high pH values (N-sodium hydroxide and higher concentrations). These reactions are characteristic of pterins (Becker & Schöpf, 1936).

Fig. 2. The absorption spectrum of the pterin-like pigment extracted from locust eyes. ---, In phosphate buffer pH 7; - · - · -, in 0.1N-NaOH; —, in glacial acetic acid.

If an aqueous solution of the pigment was chromatographed on a column of alumina (Spence, Grade 'O'), through which water had previously been run, the pigment was adsorbed as an orange-brown zone at the top of the column which could be eluted with 0.1N-sodium hydroxide, glacial acetic acid or 0.5N-ammonium hydroxide; during development of the column with ammonia solution the orange-brown zone fluoresced bright yellow. The pigment was adsorbed as an orange-yellow band showing bright yellow fluorescence when a solution in methanol containing 0.1% (v/v) of conc. hydrochloric acid was chromatographed on a column of alumina previously treated with the solvent. These chromatographic observations also indicate the presence of a pterin (Becker & Schöpf, 1936).

The above evidence indicates that the pigment under investigation was, in all probability, a pterin, but owing to the very small amounts of material available, complete identification was not possible. All that can be said is that the properties which have been observed indicate that the pigment more closely resembles xanthopterin than any other re-

ported pterin. In fact, in only two cases is there any divergence in properties, the absorption spectra and the murexide test. Our pigment exhibits a short-wave band with maxima varying from 278 to 292 m μ ., according to the pH of the solvent, whilst the consensus of recorded data indicates that the xanthopterin band occurs within the range 255–270 m μ . (see Jacobson & Simpson, 1946); however, Schöpf, Becker & Reichert (1939) have reported the maximum to lie between 275 and 284 m μ . Further, xanthopterin exhibits a band at 375–380 m μ . with an intensity 0.27 of that of the 255–270 m μ . band, whilst our pigment shows only a very wide plateau in this region with about the same relative intensity. A very small band at about 345 m μ . is, however, apparent when phosphate buffer pH 7 is used as solvent. In the murexide test, xanthopterin gives a positive reaction whilst ours did not.

The presence of a fluorescent pigment, not riboflavin but considered to be a pterin, has previously been reported in the eyes of *Schistocerca* by Busnel &

Drilhon (1942). On the information available it appears to be different from our pigment, but precise comparison is not possible because Busnel & Drilhon do not describe their methods of extraction and isolation. Pterins have, however, been reported in the eyes of a number of animals including crustaceans, fish, amphibians and reptiles (see Pirie & Simpson, 1946) but not, as yet, in any other insect species.

SUMMARY

1. The presence of a flavin, almost certainly riboflavin, has been observed in the newly laid eggs of the African migratory locust (*Locusta migratoria migratorioides* R. & F.) and of the desert locust (*Schistocerca gregaria* Forsk.).

2. The yellow fluorescent pigment occurring in the eyes of both species has been identified as a pterin; it closely resembles xanthopterin.

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REFERENCES

- Becker, E. & Schöpf, C. (1936). *Liebigs Ann.* **524**, 124.
 Bodine, J. H. & Fitzgerald, L. R. (1947). *Physiol. Zool.* **20**, 146.
 Busnel, R. G. & Drilhon, A. (1942). *Arch. Zool. exp. gén.* **82**, 321.
 Goodwin, T. W. (1949). *Biochem. J.* **45**, 472.
 Goodwin, T. W. & Srisukh, S. (1949). *Biochem. J.* **45**, 263.
 Goodwin, T. W. & Srisukh, S. (1950). *Biochem. J.* **47**, 549.
 Goodwin, T. W. & Srisukh, S. (1951). *Biochem. J.* **48**, 199.
 Gourevitch, A. (1937). *Bull. Soc. Chim. biol., Paris*, **19**, 125.
 Jacobson, W. & Simpson, D. M. (1946). *Biochem. J.* **40**, 3.
 Pirie, A. & Simpson, D. M. (1946). *Biochem. J.* **40**, 14.
 Polonovsky, M. & Busnel, R. G. (1949). *1st Int. Congr. Biochem. Abstr.* p. 72.
 Schöpf, C., Becker, E. & Reichert, R. (1939). *Liebigs Ann.* **539**, 156.

Biochemistry of Locusts

7. A NOTE ON THE EFFECT OF BREEDING TEMPERATURE ON THE CAROTENOID CONTENT OF LOCUSTS (THE AFRICAN MIGRATORY LOCUST *LOCUSTA MIGRATORIA MIGRATORIoides* R. & F. AND THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* FORSK.)

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Variations in the temperature at which locusts are reared have a profound effect on their appearance, low temperatures (28°) producing very dark insects, and high temperatures (40°) very pale insects (Parker, 1928; Husain & Mathur, 1936; Jones, 1950). The Anti-Locust Research Centre kindly placed at our disposal a number of specimens of *Locusta migratoria migratorioides* R. & F. and *Schistocerca gregaria* Forsk., reared at different temperatures, and these have been examined quantitatively for insectorubin and for the caro-

tenoids, β -carotene and astaxanthin. The results obtained for insectorubin were incorporated into the general report on the distribution of this pigment in solitary and gregarious locusts (Goodwin, 1950). The corresponding report on carotenoid distribution had, however, already been published (Goodwin, 1949) when these insects became available; consequently, for the sake of completeness, the results on the carotenoid distribution in locusts bred at different temperatures are now reported in this note.