migratoria migratorioides R. & F.) and in the eyes of *Drosophila melanogaster* (wild) and *Ephesia kühniella* have been investigated.

2. The pigments cannot be distinguished from each other. As the redox pigments reported in other insects also appear indistinguishable from our pigment, it is proposed that this widespread insect pigment be termed insectorubin.

3. Insectorubin has been isolated as a reddish-brown powder which quickly changes into an intractable dark-brown powder, reminiscent of melanin; its properties have been fully recorded.

4. It is extremely resistant to normal methods of chemical attack, but it has yielded two pyrrole derivatives. These have not been unequivocally

identified but appear to be *N*-methylpyrrole and haemopyrrole.

5. Insectorubin is the pigment responsible for the colour of the pink faeces produced by starved locusts and of the pink patches in the subcutaneous tissue and integument of *Locusta* infected with *Plistophora*.

6. Spectral data on the colours produced by a number of indole and pyrrole derivatives with Ehrlich's diazo reagent are recorded.

We are grateful to the Anti-Locust Research Centre for supplying material and for financially assisting this investigation; to Dr R. J. Beer for samples of pyrrole and indole derivatives; to Prof. C. Rimington for a helpful discussion; and to Prof. R. A. Morton, F.R.S., for advice and encouragement at all times.

REFERENCES

- Beadle, G. W. (1945). *Chem. Rev.* **37**, 15.
 Becker, E. (1939). *Biol. Zbl.* **59**, 597.
 Becker, E. (1941). *Naturwissenschaften*, **29**, 237.
 Becker, E. (1942). *Z. indukt. Abstamm.- u. Vererb. Lehre.* **80**, 157.
 Bentley, R. (1949). *Rep. Progr. Chem.* **45**, 239.
 Chauvin, R. (1938). *C.R. Acad. Sci., Paris*, **207**, 1018.
 Chauvin, R. (1941). *Ann. Soc. ent. Fr.* **110**, 133.
 Chauvin, R. (1944). *Bull. Soc. zool. France*, **69**, 154.
 Ellinger, P. & Abdel Kader, M. M. (1949). *Biochem. J.* **44**, 285.
 Ephrussi, B. (1942). *Cold Spr. Harb. Sym. quant. Biol.* **10**, 40.
 Ephrussi, B. & Herold, J. L. (1944). *Genetics*, **29**, 148.
 Fischer, H. & Orth, H. (1937). *Die Chemie des Pyrrols*, 1. Leipzig: Akademische Verlagsgesellschaft.
 Goodwin, T. W. (1950). *Biochem. J.* **47**, 544.
 Goodwin, T. W. & Srisukh, S. (1949). *Biochem. J.* **45**, 263.
 Karrer, P. & Benz, J. (1936). *Helv. chim. Acta*, **19**, 1208.
 Maas, W. K. (1948). *Genetics*, **33**, 177.
 Mason, H. S. (1948). *J. biol. Chem.* **172**, 83.
 Metcalf, R. L. (1945). *Ann. ent. Soc. Amer.* **38**, 397.
 Okay, S. (1948). *Comm. Fac. Sci. Univ. Ankara*, **1**, 178.
 Pryor, M. G. M. (1940). *Proc. roy. Soc.* **128 B**, 378, 393.
 Srisukh, S. (1950). (Unpublished observations.)
 Tatum, E. L. & Beadle, G. W. (1942). *Growth*, **6**, 27.
 Villee, C. A. (1947). *Genetics*, **32**, 277.
 Wald, G. & Allen, G. (1946). *J. gen. Physiol.* **30**, 41.

Biochemistry of Locusts

4. INSECTORUBIN METABOLISM IN THE DESERT LOCUST (*SCHISTOCERCA GREGARIA* FORSK.) AND THE AFRICAN MIGRATORY LOCUST (*LOCUSTA MIGRATORIA MIGRATORIOIDES* R. AND F.)

By T. W. GOODWIN

Department of Biochemistry, The University of Liverpool

(Received 25 May 1950)

The major pigment components of locusts are melanin (Chauvin, 1941), the carotenoids β -carotene and astaxanthin (Goodwin & Srisukh, 1948, 1949), and a redox pigment, insectorubin (Goodwin & Srisukh, 1950b). Before the general distribution of insectorubin in insects was appreciated Chauvin (1941) had termed this pigment acridioxanthin and, occasionally, acridioerythrin.

As solitary and gregarious locusts are characterized by marked colour differences (see, for example, Uvarov, 1947) it was obviously important, in investigations designed to throw light on the problem of swarming, to consider pigment metabolism in

solitary and gregarious locusts under different conditions. Such an investigation concerning the carotenoids has already been described (Goodwin, 1949) and the present report deals with insectorubin.

This pigment is a pyrrole derivative of undetermined structure which has not yet been obtained crystalline owing to the rapidity with which, in the solid state, it polymerizes into an amorphous powder, reminiscent in some ways of a melanin (Goodwin & Srisukh, 1950b). It can, however, be satisfactorily characterized spectroscopically for, when fresh, it exhibits a characteristic spectrum according to whether it is in its reduced or oxidized form.

EXPERIMENTAL

Insects. The methods of breeding the locusts, their dissection, and the removal of carotenoids have been previously described (Goodwin, 1949). The head and pronotum, body, legs, and (in adults) wings were examined separately.

Extraction of pigment. The tissue was ground finely with silver sand (acid-washed) and extracted with acetone to remove carotenoids; the carotenoid-free residue was then extracted with successive portions of cold ethanol containing 1% (v/v) of conc. HCl until the final extract was colourless; four extractions were generally sufficient. If the residue was then further extracted with methanol containing 1% (v/v) of conc. HCl a further amount of insectorubin was extracted.

It should be noted that if, between extracting the insect tissues with acetone and acid ethanol, an extraction with 80% (v/v) aqueous ethanol is interpolated, a very small amount of a yellow pigment is obtained (Goodwin & Srisukh, 1950*b*). If this extraction is omitted, the pigment is extracted along with insectorubin by acid ethanol. As the amount of yellow pigment present never contributes more than 1–2% to the total absorption at 495 m μ . of the acid methanol fraction, it was not thought necessary to separate it from insectorubin in the routine examination of the tissues for this pigment.

	Wt. of part of insect (g.)	$E_{1\text{ cm.}}^{1\%}$ (concn.)	$E_{1\text{ cm.}}^{1\%} \times \text{wt.} \times 10^3$ (amount)
Body	0.248	0.191	47.3
Head and pronotum	0.105	0.287	30.1
Legs	0.115	0.188	21.6
Wings	0.021	0.230	4.8
Total	0.489	—	103.8 (= amount/insect)
Weighted mean $E_{1\text{ cm.}}^{1\%} = \frac{\Sigma(E_{1\text{ cm.}}^{1\%} \times \text{wt.})}{\Sigma(\text{wt.})} = \frac{0.1038}{0.489} = 0.212$.			

Insectorubin is a redox pigment, being orange-yellow when oxidized and wine-red when reduced; as it was of obvious importance to determine the amounts of the two forms occurring naturally, the solvents used for extraction had to be without any oxidizing action on the pigment. It was found that reduced insectorubin was oxidized by ethanol and, to a much greater extent, by methanol unless these solvents were purified; purification was carried out by refluxing with Zn dust and solid KOH for 3 hr. followed by distillation. The solvents remained sufficiently pure for a number of weeks if kept in a tightly corked bottle in the ice chest. Reduced insectorubin dissolved in such purified solvents is stable for several hours.

The spectrographic determination of the amounts of reduced and oxidized insectorubin in a mixture of the two. For the purpose of determining the amounts of the two forms present in acid-methanol or acid-ethanol extracts a spectrographic method was devised based on that of Goodwin & Morton (1946) for determining tyrosine and tryptophan in proteins. Reduced and oxidized insectorubin have an isosbestic point at 495 m μ . (Goodwin & Srisukh, 1950*b*), i.e. the extinction of a solution at this wavelength gives directly a measure of the amount of insectorubin present irrespective of its degree of oxidation or reduction. At any other wavelength, however, the extinction will vary according to the constitution of the mixture. Examination of a large number of samples has shown that, on a scale such that the extinction E at 495 m μ . is unity, the extinctions at 450 m μ . for completely oxidized

and completely reduced solutions of insectorubin are 1.76 and 0.90, respectively. Using these values, the amounts of reduced and oxidized insectorubin in mixtures of the two can be calculated in the following manner:

Let x be the observed ratio $E_{450\text{ m}\mu.}/E_{496\text{ m}\mu.}$. Then the percentage of oxidized insectorubin in the sample is given by $100(x - 0.9)/(1.76 - 0.9)$.

Quantitative determination of insectorubin distribution in locusts. By measuring the $E_{495\text{ m}\mu.}$ value of the insectorubin extracts, $E_{1\text{ cm.}}^{1\%}$ 495 m μ . values could be determined with reference to the fresh weight of tissue. As accurate $E_{1\text{ cm.}}^{1\%}$ values are not available for pure insectorubin, owing to its rapid change in spectrum after isolation in the solid state (Goodwin & Srisukh, 1950*b*) the relative amounts of insectorubin present in various tissues were obtained by multiplying the appropriate $E_{1\text{ cm.}}^{1\%}$ values (a measure of concentration) by the weight of the tissue. As this procedure normally produced unwieldy decimal values these were multiplied by 10^3 for simplicity of presentation. As $E_{1\text{ cm.}}^{1\%}$ values were most often determined for different parts of the insects, a weighted mean $E_{1\text{ cm.}}^{1\%}$ value was calculated for the whole insect, and from this the relative amount of pigment in the whole insect. A similar method was used when carotenoid metabolism in locusts was investigated (Goodwin, 1949), but an example will make the calculation clear:

RESULTS

As stated above, extraction with acid ethanol does not remove all the insectorubin from locust tissues, for a further fraction can be obtained if the residue is treated with acid methanol. Table 1 indicates

Table 1. *The percentage of the Locusta insectorubin which is extractable only by methanol containing 1% (v/v) of concentrated hydrochloric acid*

Stage	No. of batches examined	Percentage
1st unfed	1	40.5
2nd fed	1	37.0
3rd fed	3	41.5
4th fed	1	41.9
5th fed	30	32.2
Immature adult, M	3	38.8
Immature adult, F	1	37.8
Mature adult, M	4	44.2
Mature adult, F	10	35.6
Weighted mean	—	36.9

relative amounts of these two fractions in *Locusta* at various stages of development. It will be seen that throughout life the relative amounts remain constant, the acid-methanol fraction representing on the

average 37 % of the total insectorubin present. Similar results were obtained with *Schistocerca*, and are not reported in detail here; the mean percentage of insectorubin extracted by acid methanol in this species was 40.5.

Distribution of insectorubin during locust development. A considerable amount of information has been accumulated and analysed, but only summarizing Tables are presented in this report. The presentation may be simplified in this way because it was found that there were no significant differences in the concentration of insectorubin in different parts of locusts; the relative amounts of pigments in the parts merely reflecting their relative weights. Therefore in the Tables only the data calculated for whole insects have been included.

It should be noted that in the Tables which follow the amounts and concentrations of insectorubin recorded refer only to the portion of the pigment soluble in acid ethanol; these values, however, bear a constant ratio to the total amount of pigment present at any developmental stage (see above), so that they can be taken as a measure of developmental variation in total insectorubin production.

In Tables 2 and 3 are shown mean values for both the concentration and amount of insectorubin in

Fig. 1. The variations in the concentration and amount of insectorubin in the developing locust (data for *Schistocerca* and *Locusta* combined). The vertical lines indicate the standard deviation of the results.

gregarious *Locusta* and *Schistocerca*. It will be seen that insectorubin exists in the newly hatched, unfed hoppers at a concentration which remains practically

Table 2. *The concentration and the amount of acid-ethanol-soluble insectorubin in developing gregarious Locusta*

Stage	No. of insects examined	No. of batches examined	$E \frac{1}{100} \text{ cm. } 495 \text{ m}\mu.^*$	Amount†/insect
1st unfed	143	3	0.106	1.7
1st fed	307	4	0.122	1.7
2nd fed	100	3	0.082	3.1
3rd fed	69	4	0.115	8.8
4th fed	38	4	0.091	15.9
5th fed	42	11	0.113	69
Immature M	23	9	0.075	80.0
Immature F	32	11	0.063	71.1
Mature M	39	11	0.039	42.5
Mature F	21	12	0.053	91.8
Immature F (velvety-blue)	4	4	0.077	61.8
<i>Locusta</i> infected with <i>Plistophora</i>				
Mature M (heavily infected)	4	2	0.032	48.9
Mature F (heavily infected)	5	5	0.032	87.9
Mature F (lightly infected)	2	2	0.047	115

* $E \frac{1}{100} \text{ cm.}$ values used as measures of concentration. † See text for method of calculating amounts.

Table 3. *The concentration and amount of acid-ethanol-soluble insectorubin in developing gregarious Schistocerca*

Stage	No. of insects examined	No. of batches examined	$E \frac{1}{100} \text{ cm. } 495 \text{ m}\mu.^*$	Amount†/insect
1st unfed	56	2	0.082	1.5
1st fed	154	3	0.082	1.9
2nd fed	29	2	0.139	7.2
3rd fed	44	4	0.117	14
4th fed	22	3	0.111	29
5th fed	12	2	0.079	53.8
Immature M	12	5	0.090	104
Immature F	11	5	0.114	138
Mature M	9	6	0.067	91
Mature F	6	4	0.061	134

* $E \frac{1}{100} \text{ cm.}$ values are taken as measures of concentration. † See text for calculation of amount.

constant throughout the five hopper instars; in the immature adults there is a marked tendency for the concentration to fall and in the sexually mature insects this becomes much more pronounced. These changes are very much the same in the two species so that data are combined in Fig. 1 which emphasizes the changes and also shows the variations in the amount of insectorubin produced during the development of the insects.

It will also be noted from Table 2 that the very dark velvety-blue gregarious *Locusta*, which are occasionally encountered, have normal insectorubin contents although their carotenoid metabolism is unique (Goodwin, 1949). Infection with the protozoan *Phistophora* has also no effect on the insectorubin although carotenoid metabolism was deranged (Goodwin, 1949). It is also to be noted that between the sexes no differential insectorubin distribution is apparent.

Insectorubin in the dorsal and ventral regions of Locusta. The gregarious *Locusta* hoppers can be divided quite sharply into two colour regions; the dorsal region, which is black, and the ventral region which is orange-brown. Although the extraction of insectorubin does not alter the external colour of these regions to any marked extent, it was considered important to know whether the pigment accumulated in the darker region. It will be seen from Table 4 that insectorubin does accumulate in the dorsal regions not only of the gregarious hoppers and adults but also of solitary hoppers which are bright green dorsally and light greyish green ventrally (see below). Owing to the different weights of the dorsal and ventral samples, however, the amount of pigment present is approximately the same in both regions.

Insectorubin production in the developing locust egg. Pigment production has been followed during the embryonic development of both *Locusta* and *Schistocerca*. The results obtained were very similar for both species and the combined data have been incorporated into Fig. 2, which shows clearly that the pigment is produced during the incubation period. The relative amounts of insectorubin per egg were calculated from the expression $E \times v \times 10^3/p$, where E is the extinction at 495 m μ . of a v ml. acid-ethanolic extract of p eggs.

Fig. 2. The production of insectorubin in the developing locust egg.

Effect of temperature on insectorubin production. The amounts of insectorubin produced at various stages by *Schistocerca* reared at different temperatures are recorded in Table 5. It is clearly demonstrated that pigment production is increased at low temperatures and diminished at high temperatures. Increasing the breeding temperature from its usual

Table 4. *Insectorubin distribution in dorsal and ventral regions of 5th instar Locusta*

Phase	No. examined	Dorsal region				Ventral region			
		Acid-ethanolic fraction		Acid-methanolic fraction		Acid-ethanolic fraction		Acid-methanolic fraction	
		$E_{1\text{ cm.}}^{1\%}$	Amount	$E_{1\text{ cm.}}^{1\%}$	Amount	$E_{1\text{ cm.}}^{1\%}$	Amount	$E_{1\text{ cm.}}^{1\%}$	Amount
Gregarious	2	0.147	32	—	—	0.072	35	0.028	13
	2	0.089	22	—	—	0.044	22	—	—
	2	0.099	18	0.046	8	0.041	17	0.028	11
Solitary (green)	2	0.175	15	0.084	7	0.107	21	0.044	9

Table 5. *The effect of breeding temperature on the production of insectorubin in Schistocerca*

Instar	Temperature											
	28°			35°			40°			28° day, 40° night		
	No. ex- amined	$E\frac{1}{\text{cm.}}$	Amount/ insect	No. ex- amined	$E\frac{1}{\text{cm.}}$	Amount/ insect	No. ex- amined	$E\frac{1}{\text{cm.}}$	Amount/ insect	No. ex- amined	$E\frac{1}{\text{cm.}}$	Amount/ insect
1st fed	1	0.133	2.6	2	0.082	1.5	1	0.073	0.8	—	—	—
5th fed	4	0.325	503	2	0.079	54	4	0.015	9.75	2	0.055	30.4
Immature adult	11	0.418	495	10	0.059	122	2	0.011	10	2	0.018	37
Mature adult	—	—	—	10	0.052	110	2	0.018	29	—	—	—

value of 35° to 40° almost completely prevents insectorubin synthesis and produces a very pale insect; conversely lowering of the breeding temperature causes a threefold increase in pigment production and the locusts are very black. When insects are kept partly at 28° (day) and partly at 40° (night) the two effects tend to balance each other out. Similar experiments carried out on a smaller scale with *Locusta* gave identical results and are not reported in detail here.

Relative amounts of oxidized and reduced insectorubin in developing locusts. The percentage of each form present was determined in a number of locusts at every stage of development according to the method outlined above. Results obtained using both *Schistocerca* and *Locusta* were not significantly different and are combined in Table 6. It will be seen that on the average 14.5% of the extracted pigment was in the oxidized form; no significant differences in the percentage of oxidized form in the various stages could be demonstrated.

Table 6. *The percentage of oxidized insectorubin in developing locusts*

Stage	No. of batches examined	Oxidized pigment (%)
1	3	23.4
2	4	26.8
3	7	11.5
4	3	4.3
5	3	14.7
Immature adult, M	8	6.9
Immature adult, F	13	11.0
Mature adult, M	10	19.4
Mature adult, F	11	22.2
Mean (%)	—	14.5

Excretion of insectorubin in exuviae. In passing from one instar to the next, locusts, in common with most insects, cast off their skins. These moults (exuviae) have been examined for insectorubin. It has been found that the pigment exists in the exuviae at a concentration about 40% of that in the insect which shed them. Owing to the small weights of the exuviae, however, they only deplete the hopper to the extent of about 5% of its pigment. The exuviae of buff solitary *Locusta* have been examined in some detail and the results obtained are collected in Table 7.

During moulting, locusts undergo typical colour changes; at the moment of moulting the insect is quite pale in colour and only darkens after about 1 hr.; it was thought that changes in insectorubin, possibly consistent with its transformation into melanin, might be observed. Accordingly, a number of similarly reared *Locusta* fourth instar hoppers were examined during ecdysis. The results (Table 8) show that no significant differences were apparent in the insectorubin content just before, during, and just after ecdysis.

Table 8. *The insectorubin content of Locusta at different periods during the 4th–5th instar moult*

(Five insects examined at each stage.)

Stage of moult	$E\frac{1}{1\text{cm.}}$	Amount of insectorubin/locust
A 1st sign that moulting is imminent—wagging of abdomen	0.088	19.3
B Skin split along middorsal line	0.063	16.4
C Hoppers completely out of old skins, but still light-coloured and very soft	0.071	15.3
D Hoppers out of old skin and with normal coloration (i.e. about 1 hr. after C)	0.077	16.9

Insectorubin in solitary locusts. Solitary locusts can be roughly divided into two types according to whether they are predominantly buff or predominantly green. The insectorubin content of fifth instar *Locusta* specimens of these types are recorded in Table 9; also recorded are the values obtained for a group of fifth instar gregarious hoppers reared at the same time and of members of this group which were converted into green solitaries by isolating them at the third and fourth instar, respectively. It will be seen that very little difference is apparent in the insectorubin content of gregarious or solitary hoppers when produced under the same conditions; further, as might be expected, no differences were found in newly hatched *Locusta* bred from solitary and gregarious parents respectively.

Schistocerca solitaries differ from *Locusta* solitaries in one important respect. It was found that the

Table 7. *Distribution of insectorubin in locust exuviae*

Type	Stage	Wt. of one moult (g.)	No. of moults examined	$E\frac{1}{1\text{cm.}}$	Amount/moult
<i>Locusta</i> solitary	1st→2nd	0.000589	46	0.216	0.116
	2nd→3rd	0.00139	38	0.0858	0.119
	3rd→4th	0.00281	30	0.0447	0.126
	4th→5th	0.00461	44	0.0444	0.205
	5th→adult	0.0205	24	0.0598	1.07
<i>Locusta</i> gregarious	Mixed	0.0200	—	0.0470	—
<i>Schistocerca</i> gregarious	Mixed	0.0247	—	0.0413	—

Table 9. *Insectorubin content of solitary Locusta*

Material	No. examined	Acid-ethanol-soluble fraction		Acid-methanol-soluble fraction	
		$E_{1\text{ cm.}}^{1\%}$	Amount	$E_{1\text{ cm.}}^{1\%}$	Amount
1st instar. Progeny of gregarious insects	143	0.106	1.7	—	—
1st instar. Progeny of solitary insects	16	0.114	2.0	—	—
5th instar. Typical gregarious insects	4	0.156	95	0.088	45
5th instar. Solitaries (colours varying from buff→grey→green)	5	0.165	110	0.069	—
5th instar. Green crowded until 4th instar then isolated	5	0.167	103	0.071	37
5th instar. Green crowded until 3rd instar then isolated	5	0.147	92	0.069	45
5th instar. Solitary buff→grey	2	0.144	99	0.057	40

green type contains little or no insectorubin, the little that is present being apparently located in the eyes; buff *Schistocerca* solitaries contain normal amounts of insectorubin, e.g. the mean values for a number of solitary fifth stage buffs were $E_{1\text{ cm.}}^{1\%}$ 0.102 and amount 90.

DISCUSSION

According to Chauvin (1941) insectorubin (acridioxanthin) is an anthocyanin plant pigment, which is absorbed by locusts and stored mainly in the integument. This view is now known to be mistaken; insectorubin is a pyrrole derivative typical of insects (Goodwin & Srisukh, 1950b) which in the case of *Drosophila* and *Ephesia* can be synthesized from tryptophan via α -hydroxytryptophan and kynurenine (see, for example, Beadle, 1945). From the results shown in Fig. 2 there can be no doubt that insectorubin can be synthesized from some colourless precursor in the developing locust egg and that an external source of the pigment is not necessary. The precursor of insectorubin in locusts has not yet been determined, but it should be borne in mind that compounds other than tryptophan (e.g. pyrrole residues from chlorophyll or glycine) may also be precursors. Further evidence indicating that insectorubin is an endogenous pigment comes from the fact that, in spite of Chauvin's assertion to the contrary, all attempts in this laboratory to detect it in green plants have failed.

Dealing only with *Schistocerca*, Chauvin (1941) outlined the following insectorubin cycle: the small amounts of insectorubin present at birth increases progressively as food is eaten until the beginning of the adult stage when insectorubin is excreted and no further pigment is laid down; at sexual maturity the locusts possess very little pigment, for the hypodermal cells alter (histologically) and become incapable of fixing new 'anthocyanin' or retaining that which they already possess; the pigment is excreted in its reduced form during adult life. No quantitative data are presented to support these views. Although Chauvin used only *Schistocerca* our

experience indicates that gregarious *Schistocerca* and *Locusta* are identical as regards insectorubin metabolism, and in discussing the insectorubin cycle our comments can apply equally to both species.

The results obtained during the present investigation (Tables 2 and 3; Fig. 1) demonstrate the gradual increase in insectorubin during successive instars and thus confirm part of Chauvin's statement. It should be noted that the increase in total quantity follows a typical growth curve because the concentration of the pigment does not alter during hopper life. The results obtained after adult emergence indicate that the second part of Chauvin's statement is inexact in detail. No further pigment is laid down after emergence, but sexually mature insects still contain appreciable amounts. A decrease in concentration does, however, occur after emergence, but this is mainly owing to the increased size of mature insects, the net result being that the amount of pigment remains practically constant during adult life. With regard to excretion of insectorubin during adult life, it has not been possible to demonstrate its presence in normal faeces although it is present in the pink faeces which are sometimes excreted (Goodwin & Srisukh, 1950b). These pink faeces are, however, only produced during starvation, but the occurrence of insectorubin in them does indicate that locusts possess a mechanism by which they can excrete the pigment.

The marked change in insectorubin metabolism at adult emergence, which is rather similar to that of astaxanthin (Goodwin, 1949) can best be accounted for in one of two ways: (a) synthesis continues at a reduced rate so that it only just balances the physiological destruction or utilization of the pigment, or (b) synthesis continues at the pre-emergence level, but rate of destruction or utilization is increased; it seems unlikely that synthesis abruptly ceases, because if so one would expect a gradual disappearance of pigment during adult life.

An apparent fractionation of insectorubin can be achieved by exhaustively extracting the dried tissues first with acid ethanol and then with acid

methanol; between 30 and 40 % of the total pigment occurs in the acid-methanolic fraction irrespective of the stage of development (Table 1). The reasons for this fractionation are not yet apparent although two explanations are possible; (a) the insectorubin is attached to two different protein carriers, one complex being more easily broken down than the other, or (b) one fraction consists of insectorubin in a more 'aged' (polymerized) form which would be more soluble in acid methanol than in acid ethanol (Goodwin & Srisukh, 1950*b*).

Insectorubin and the colour of Locusta. It is appropriate at this point to consider how far the present investigation and those earlier in the series (Goodwin, 1949; Goodwin & Srisukh, 1949, 1950*b*) help in the explanation of locust coloration. In this we are concerned only with the general appearance of locusts and not with the details of the colour patterns, which are important in the recognition of the phases. The Anti-Locust Research Centre laboratory (Jones, 1950) has confirmed that locusts reared at high temperature are pale and at low temperature dark (Husain & Mathur, 1936); the present work has demonstrated the absence of insectorubin from pale locusts and the presence of unusually large amounts in the dark ones (Table 5). This tempts one to conclude that the amount of insectorubin present is mainly responsible for the colour of the locust. The validity of this conclusion depends on the development stage of the locust; in adults (except sexually mature males) this is true because the colour pattern is due almost entirely to insectorubin (Goodwin & Srisukh, 1950*a*). In hoppers (fifth instar insects are considered here but the discussion applies to other stages) insectorubin plays little or no part in the external coloration. The dorsal regions of a *Locusta* hopper are black and the ventral regions orange-brown. If these two regions are extracted separately the dorsal regions yield more insectorubin (Table 4), but the tissues remain quite black and orange-brown respectively (Goodwin, 1950). The major part of the coloration is thus due not to insectorubin but to insoluble black and orange-brown pigments. The black pigment has all the attributes of a typical melanin (Chauvin, 1939), whilst the brown regions may be due to a very weak and scattered melanization. Whether this pigment is melanin in its usually accepted sense or is the result of the 'tanning' of the cuticle proteins (Pryor, 1940; Hackman, Pryor & Todd, 1948), is of no importance for this discussion.

The insectorubin which occurs in the hypodermal cells (Chauvin, 1941; Daniel, 1949) is therefore not externally visible in hoppers because of the presence of cuticular melanin; in immature males and females and mature females, melanin disappears from the cuticle (Chauvin 1941; Goodwin & Srisukh, 1950*a*) and insectorubin, either free or attached to a protein,

plays a major part in the external pigmentation of the insects. In sexually mature males β -carotene migrates to the cuticle, masks to some extent the insectorubin and the insects appear yellow. The same type of considerations can be applied to *Schistocerca*, except that it should be noted that the yellow patches in fifth stage hoppers are probably due to cuticular β -carotene. In adult *Schistocerca* small amounts of melanin seem to occur only in the elytra (Uvarov, 1948), whilst in *Locusta* there are black bands on the pronotum and the inside of the femur.

The variation in coloration of hoppers bred at different temperatures is thus due to the production of varying amounts of melanin, a parallel variation in insectorubin production having very little effect on coloration; at adult emergence, however, when melanin almost entirely disappears from the cuticle, the degree of coloration is then controlled, except in sexually mature males, by the amount of insectorubin present. This interpretation is borne out by the observations on moulting hoppers (Tables 7 and 8). Immediately after moulting, although only very little insectorubin is lost, the insect is light coloured; no insectorubin is visible through the very light-buff opaque soft cuticle; the coloration only appears after the formation of melanin or the tanning of the cuticle.

The similar dependence of melanin and insectorubin on temperature could indicate that they are metabolically interrelated, and this is supported by the observation of Goodwin & Srisukh (1950*b*), that isolated insectorubin rapidly changes into a brown melanin-like powder; against this, however, must be considered the disappearance of melanin, but not of insectorubin, from immature adults and from solitary *Locusta* hoppers (see below).

Insectorubin in solitary locusts. Solitary *Locusta* hoppers, whether green or buff, contain approximately the same amounts of insectorubin as do gregarious hoppers, in spite of the great difference in colour (Table 9). Similar results are obtained if gregarious hoppers are kept crowded until the third or fourth instar and then segregated. The typical green coloration of the dorsal aspects appears, but the insectorubin content remains unchanged. Thus, as in gregarious hoppers, insectorubin plays no major part in the coloration of solitary hoppers. Melanin disappears from the cuticle and its place is taken by green insectoverdin, a complex of a glaucobilin and a caroteno-protein (Junge, 1941; Goodwin & Srisukh, 1950*a, c*), although the amount of carotenoids present does not alter (Goodwin, 1949). Apparently, on isolation, a hopper gradually loses its cuticular melanin which is replaced by insectoverdin. It would be reasonable to suppose that the major change in colour takes place at ecdysis, but precise observations on this point have not yet been made. As in the green hoppers, insectorubin appears

to play no great part in the coloration of buff hoppers, but the pigment responsible for the buff colour has not yet been characterized. The same considerations apply to solitary *Schistocerca* with the one important difference that green *Schistocerca* solitaires contain very little insectorubin. It is appropriate to note here that the haemolymph of solitaires is invariably green, whilst that of gregarious hoppers is almost always golden yellow (Goodwin & Srisukh, 1950c).

Apart from the colour variation, the main difference between solitary and gregarious locusts is in their relative activities, the gregarious type being much more active (Uvarov, 1948). This increased activity results in slightly increased internal temperatures (Volkonsky, 1939); exposure to sunlight has the same effect, the rise in the temperature of gregarious (black) hoppers being, as might be expected, higher than in the solitary greens (Strel'nikov, 1936). Thus under the same environmental conditions it would be reasonable to expect solitaires to be at a somewhat lower body temperature than gregarious locusts. If temperature were the only consideration, then from our laboratory results solitary hoppers would be expected to contain more insectorubin and melanin; what is observed is that the insectorubin content either remains constant (*Locusta*) or disappears (*Schistocerca*) whilst, owing to a qualitative change in metabolism, melanin disappears and is replaced by insectoverdin. Obviously, increases in metabolic activity connected with changes of temperature may have little if any effect on the colour changes produced in passing from gregarious to solitary phases and another factor, perhaps not concerned at all with metabolic activity, must be sought.

Faure (1932) has suggested that phase differentiation in locusts is concerned with the accumulation or disappearance of a hypothetical substance called 'locustine'. None of the materials examined in the present investigation has the properties required of 'locustine'. No excessive pigment formation occurs in solitary insects. There is, however, an inhibition of melanin production (as well as in the case of green *Schistocerca* of insectorubin); the appearance of insectoverdin in place of melanin is characteristic of the solitary phase.

The different colours which insectorubin assumes in its oxidized and reduced states (orange-yellow and wine-red, respectively) were possible factors in the colour changes observed in developing locusts, but Table 6 indicates that no significant difference in the relative amounts of the two forms occurs during development. The yellow colour of sexually mature males, *Locusta* and *Schistocerca*, and of the yellow regions of fifth stage *Schistocerca* is in fact due to carotenoid pigments (Goodwin & Srisukh, 1950a).

SUMMARY

1. The formation of insectorubin has been studied in the solitary and gregarious phases of two locust species: *Locusta migratoria migratorioides* R. and F. and *Schistocerca gregaria* Forsk.

2. Differential accumulation of insectorubin does not occur in the head and pronotum, body, legs and wings, and no sexual or species differentiation is seen; but a higher concentration occurs in the dorsal than in the ventral regions.

3. During development, insectorubin concentration is constant throughout the hopper stages, but falls during adult life. The amount of pigment present increases up to adult emergence; thereafter it either remains constant or falls slightly.

4. Certain atypical velvety-blue *Locusta* and *Locusta* infected with *Plistophora* contained normal amounts of insectorubin.

5. About 37% of the insectorubin present in locusts is not extractable with acid ethanol, but is extractable with acid methanol. About 10–15% of the pigment occurs in the oxidized form.

6. Temperature has a marked effect on insectorubin production; reduction of breeding temperature from 35° to 28° causes a threefold increased production, whilst increasing it to 40° almost completely prevents its production.

7. Insectorubin, which cannot be detected in newly laid eggs, is synthesized during the embryonic development of locusts.

8. Small amounts of insectorubin are excreted in exuviae, but no significant change in insectorubin content occurs during the moulting process.

9. Insectorubin occurs in the most common types of solitary *Locusta* (greens and buffs) in the same concentration as that found in corresponding gregarious locusts. Gregarious (black) *Locusta* hoppers converted into solitary (green) hoppers by isolation at the third or fourth instar have the same insectorubin content as hoppers crowded all their life. The change in colour is due to the disappearance of melanin and its replacement by insectoverdin. Green solitary *Schistocerca* contain only small amounts of insectorubin whilst buffs contain normal amounts.

10. The role of insectorubin in the pigmentation of solitary and gregarious locusts is discussed.

The author is indebted to the Anti-Locust Research Centre for material and a grant towards laboratory expenses. The advice and help of Dr B. P. Uvarov, F.R.S., and Dr D. L. Gunn of the Centre were always freely given and were invaluable. Thanks are also due to Prof. R. A. Morton, F.R.S., for his encouragement and advice.

REFERENCES

- Beadle, G. W. (1945). *Chem. Rev.* **37**, 15.
 Chauvin, R. (1939). *Bull. Histol. Tech. Micr.* **16**, 137.
 Chauvin, R. (1941). *Ann. Soc. ent. Fr.* **110**, 133.
 Daniel, R. J. (1949). (Personal communication.)
 Faure, J. C. (1932). *Bull. ent. Res.* **23**, 293.
 Goodwin, T. W. (1949). *Biochem. J.* **45**, 472.
 Goodwin, T. W. (1950). (Unpublished observations.)
 Goodwin, T. W. & Morton, R. A. (1946). *Biochem. J.* **40**, 628.
 Goodwin, T. W. & Srisukh, S. (1948). *Nature, Lond.*, **161**, 525.
 Goodwin, T. W. & Srisukh, S. (1949). *Biochem. J.* **45**, 263.
 Goodwin, T. W. & Srisukh, S. (1950a). *Biochem. J.* **46**, xvii.
 Goodwin, T. W. & Srisukh, S. (1950b). *Biochem. J.* **47**, 549.
 Goodwin, T. W. & Srisukh, S. (1950c). (Unpublished observations.)
 Hackman, R. H., Pryor, M. G. M. & Todd, A. R. (1948). *Biochem. J.* **43**, 474.
 Husain, M. A. & Mathur, C. B. (1936). *Ind. J. agric. Sci.* **6**, 591.
 Jones, P. H. (1950). (Unpublished observations.)
 Junge, H. (1941). *Hoppe-Seyl. Z.* **268**, 179.
 Pryor, M. G. M. (1940). *Proc. roy. Soc. B*, **128**, 378.
 Strel'nikov, I. D. (1936). *Trav. Inst. zool. Acad. Sci. U.R.S.S.* **2**, 673.
 Uvarov, B. P. (1947). *New Biology*, **3**, 9.
 Uvarov, B. P. (1948). *Trans. R. ent. Soc. Lond.* **99**, 1.
 Volkonsky, M. (1939). *Arch. Inst. Pasteur Algér.* **17**, 194.

Vitamin B₁-like Activity of 2-Amino-5-methylthiazole-4-carboxylic Acid

By G. DI MAGGIO

Pharmacological Institute, University of Catania

(Received 16 January 1950)

During the last few years investigations on the biological activity of vitamin B₁ have taken a new course, especially as a result of information obtained from studies on growth factors. Knight (1937a, b) demonstrated that it is not necessary to supply staphylococci with the entire molecule of aneurin, as they are able to satisfy their requirements when supplied with the pyrimidine and thiazole components. Many authors (see Lwoff, 1943) have established the same capability for other micro-organisms and also for higher organisms (Robbins, Bartley, Hogan & Richardson 1937). The last-mentioned workers demonstrated that a mixture of the pyrimidine and thiazole components could replace vitamin B₁ in the diet of polyneuritic pigeons. This has also been demonstrated by Abderhalden & Abderhalden (1938), who have found further (1939) that such synthesizing ability is also possessed, to a limited extent, by extracts from different organs of various animals.

It is further known that the thiazoles stimulate the growth of some organisms (flagellates, Phycomyces) in the same general way as the whole vitamin functions in vertebrates and metazoa in general. Thiazole derivatives have, however, been studied only slightly from the pharmacological standpoint (see Di Maggio, 1946). It seemed of interest therefore to inquire into the action on experimental beriberi of substances containing only the thiazole nucleus.

The present investigation concerns 2-amino-5-methylthiazole-4-carboxylic acid (I).

EXPERIMENTAL

This compound, as the Na salt, was dissolved so that 1 ml. of water contained 0.0166 mg. It was administered orally at a level of 0.0166 mg./100 g. body weight in single daily doses to adult pigeons fed only with rice (12.5 g. in the morning and 12.5 g. in the evening). The doses were given before the morning feed. The pigeons (twelve in number), weighing 450–500 g. and all of the same breed, were obtained from a private farm. Twelve other pigeons from the same farm received no thiazole treatment, but only the rice diet (12.5 g. in the morning and 12.5 g. in the evening) and were used as controls. Water was provided *ad lib.* for all birds.

The gastro-intestinal symptoms (vomiting, diarrhoea) and the reactions to exercise were followed daily. The rectal temperature was measured each day and the body weight every 3 days. Blood samples were taken every 8 days from a wing vein and sugar determined by Hagedorn and Jensen's method. In the terminal phase of the symptoms, daily blood-sugar estimations were carried out.