The Biochemistry of Locusts

2. CAROTENOID DISTRIBUTION IN SOLITARY AND GREGARIOUS PHASES OF THE AFRICAN MIGRATORY LOCUST (*LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.) AND THE DESERT LOCUST (*SCHISTOCERCA GREGARIA* FORSK)

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The tremendous destruction which can be caused by locust plagues is widely appreciated, but, although locust research has attracted the interest of a large number of workers, the biochemical aspects of the problem have received little attention.

Uvarov (1921) was the first to appreciate the significance of the different forms of *Locusta migratoria*, and his theory of phases as applied to Acrididae which swarm is now universally accepted. In the *solitaria* phase locusts live in isolation; this implies that they are independent of their neighbours rather than that they are particularly scarce. In the *gregaria* phase hoppers (nymphs) occur in dense bands, keep close to each other, and march together. Gregarious adults form vast swarms which keep together even in flight. The two extreme phases are connected by a complex series of transitional forms which are descriptively named *transiens*. Factors which are known to influence the transition from solitary to gregarious forms and vice versa have recently been discussed by Uvarov (1947). The main factor controlling phase transformation is probably the population density; when solitary locusts are brought together they gradually change into the gregarious phase which is most strikingly evidenced by the insects becoming quite different in colour. The effect of crowding is in all probability to increase the activity of hoppers owing to frequent encounters with their neighbours. This increased activity appears to be the fundamental cause of the colour changes, for a solitary hopper maintained in isolation will also assume gregarious characteristics when stimulated to continuous exertion (Husain & Mathur, 1936).

After hatching, *L. migratoria* and *Schistocerca gregaria* (the two species under present consideration) normally go through five hopper stages, moulting at the end of each, and after about 22 days (at 32°) finally emerge as winged adults; under optimal conditions, from 2 to 4 weeks after emergence the adults become sexually mature (Uvarov, 1928).

Gregarious hoppers of *L. migratoria* are characterized in the early stages by their dark appearance which is broken by yellow or brown spots and stripes; in the later stages orange brown predominates, although there are still considerable

regions of black pigmentation. At adult emergence there appears to be little black pigment and the colour is greyish brown, but on reaching maturity the males become bright yellow and the females dark brown. The overall colour pattern is very stable. The coloration of solitary *Locusta*, on the other hand, varies considerably; solitaires are generally lighter coloured than *gregaria* and tend to adopt the colour of their environment (Faure, 1932). They contain little, if any, black pigment and do not undergo colour changes at maturity.

Solitary and gregarious *Schistocerca* exhibit very much the same characteristics as do the corresponding *Locusta*, although the pigment pattern is different. The coloration of gregarious hoppers is very stable and in the later stages the insects are lemon yellow with very pronounced black patterns. Immature adults are generally pinkish with small dark spots, especially on the elytra, and they turn to yellow when maturity is reached. The colour of solitary *Schistocerca* is much more variable than that of gregarious *Schistocerca*; in general, hoppers are green, immature adults greenish white, and mature adults grey (Uvarov, 1928). Some appreciation of these colour variations in *Schistocerca* and *Locusta* can be obtained by examining the coloured plates produced by Faure (1932).

It will be obvious from the above very brief survey that investigations into the biochemistry of locust pigmentation are essential to a deeper understanding of the phase transformation. The pigments which are stated to occur in locusts are melanin (Chauvin, 1937, 1939*a*), acridioxanthin, a pigment of unknown constitution (Chauvin, 1938*a, b*, 1939*b*, 1940, 1941*a, b*, 1944), carotenoids (Lederer, 1935; Chauvin, 1941*b*; Goodwin & Srisukh, 1948, 1949), an unidentified green pigment variously described as a chlorophyll derivative (Chauvin, 1939*a*) and as a flavone (Chauvin, 1941*b*), flavins (Gourevitch, 1937; Drilhon & Busnel, 1939; Busnel & Drilhon, 1942; Drilhon, 1943), and, possibly, a pterin (Busnel & Drilhon, 1942).

In the present investigation attention has been confined to the two carotenoids, β -carotene and astaxanthin, which occur in both species (Goodwin & Srisukh, 1948, 1949), and the results of a study of

their quantitative distribution in *Locusta* and *Schistocerca* at various stages of development and in both the solitary and gregarious phases are now reported. A similar investigation on the distribution of acridioxanthin will be reported later.

MATERIAL AND METHODS

Two species of locusta have been studied: the African migratory locust (*Locusta migratoria migratorioides* R. & F.) and the desert locust (*Schistocerca gregaria* Forsk.). The locusts were bred in captivity at the Anti-Locust Research Centre in London; they were maintained under reasonably constant conditions and fed entirely on grass and wheat bran. The only difference between the breeding of the solitary and the gregarious phases was that the solitaries were completely isolated (one in a 3 lb. jam pot of capacity about 1 l.), whilst the gregarious insects were reared in crowds (about 50/jam pot or at high densities in cages up to 150 l. in capacity). Specimens were sent to Liverpool by post and generally arrived in good condition; any dead insects were discarded. In order to determine pigment distribution the insects were dissected into three portions: head (comprising the head and pronotum), body and legs; the wings, which only contain relatively small amounts of carotenoids (Goodwin & Srisukh, 1949) were not examined systematically. The insects were killed with ether, although occasionally this step was omitted, and the heads and pronota snipped off with sharp scissors; any internal organs remaining attached to the head were removed with fine forceps, as was any residual food material in the buccal cavity. The legs and wings were removed from the bodies which were then slit ventrally and the internal organs removed. The various parts were weighed within 5–10 min. of dissection in order to reduce to a minimum errors likely to arise from drying of the tissues; this is especially necessary in the case of the body.

Extraction of pigments. Each portion was ground in a mortar with acid-washed silver sand and, if necessary, a small amount of anhydrous Na_2SO_4 ; the carotenoids were then extracted with cold acetone *in situ* by stirring carefully with a pestle. The acetone was decanted and filtered through a G4 sintered-glass filter. The residue was re-extracted with acetone, and the process repeated until no colour showed in the acetone; three extractions were generally sufficient.

Chromatographic separation of the carotenoids. The acetone was removed under nitrogen, and the residue immediately dissolved in a small volume (5–10 ml.) of light petroleum (b.p. 40–60°). This solution was run through a small column (10 × 2 cm.) of defatted bone meal (Mann, 1943; Goodwin & Morton, 1946; Glover, Goodwin & Morton, 1948), and the column washed with further small quantities of light petroleum until no more pigment was eluted; this fraction consisted of β -carotene only. The astaxanthin, sometimes contaminated by traces of carotenoid oxidation products, was then eluted with acetone. The volumes of the β -carotene and astaxanthin fractions were then suitably adjusted for spectrophotometric analysis.

Spectrophotometric analysis. Measurements of light absorption were made in a Beckman quartz photoelectric spectrophotometer. The measurements of both solutions (β -carotene and astaxanthin) were made at 450 m μ . The wavelength 450 m μ . was used for both solutions in order to

reduce the time required to examine a considerable number of solutions. This wavelength is λ_{max} for β -carotene but not for astaxanthin; however, the $E_{1\text{cm.}}^{1\%}$ 450 m μ . for pure astaxanthin in acetone is known (*vide infra*).

Calculation of results. The $E_{1\text{cm.}}^{1\%}$ 450 m μ . values for the carotene and astaxanthin fractions for the various parts of the body were calculated using the expression $E_{1\text{cm.}}^{1\%} = E/cd$, where E is the observed extinction for a solution of $c\%$ in a cell d cm. thick. In order to obtain an accurate $E_{1\text{cm.}}^{1\%}$ value for the whole insect a weighted mean value of $E_{1\text{cm.}}^{1\%}$ was calculated, e.g.:

	Wt. of part (g.)	$E_{1\text{cm.}}^{1\%}$	$E_{1\text{cm.}}^{1\%} \times \text{wt.}$
Body	0.608	0.0233	0.01417
Head and pronotum	0.210	0.0193	0.00405
Legs	0.234	0.0178	0.00416
Total	1.052		0.02238

$$\text{Weighted mean } E_{1\text{cm.}}^{1\%} = \frac{\Sigma(E_{1\text{cm.}}^{1\%} \times \text{wt.})}{\Sigma \text{wt.}} = \frac{0.02238}{1.052} = 0.0213.$$

The amount of carotene or astaxanthin per insect can then be calculated using the expression:

$$\text{Amount } (\mu\text{g.}) = \frac{E_{1\text{cm.}}^{1\%} \text{ 450 m}\mu. (\text{obs.}) \times \text{wt.} \times 10^6}{E_{1\text{cm.}}^{1\%} \text{ 450 m}\mu. \text{ of pure pigment}}.$$

$E_{1\text{cm.}}^{1\%}$ 450 m μ . for pure β -carotene in light petroleum was taken as 2500 and for astaxanthin in acetone, 1968. This latter figure was obtained from the curve in acetone of spectroscopically pure astaxanthin (Goodwin & Srisukh, 1949) assuming that $E_{1\text{cm.}}^{1\%}$ (λ_{max}) in acetone is the same as that in cyclohexane, i.e. 2500 (Karrer & Würgler, 1943).

RESULTS

A very considerable mass of data has been accumulated and analysed, but in order to conserve space and simplify presentation only summarizing tables are presented. An important conclusion, which allowed the Tables to be considerably simplified, was reached when the complete data were considered. It was that there are no significant differences in the concentrations of the pigments in the body, head and pronotum, and legs, and that the relative amounts of pigment in these tissues (about 50, 25–30, and 20–25 %, respectively, of the total) merely reflect the relative weights of these parts. Because of this it has been considered unnecessary to record the results of analyses on the separate parts of the body, and only those referring to whole insects are presented and discussed.

In Tables 1–4 are collected the values for both concentrations and amounts of carotene and astaxanthin of whole insects at most stages of development of solitary and gregarious *Locusta* and *Schistocerca*.

The most important point which emerged from a study of these tables is that in both *Locusta* and

Table 1. Carotenoid distribution in gregarious *Locusta*

Stage	No. of insects examined	No. of batches examined	β -Carotene		Astaxanthin	
			$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (petrol)	Amount/insect ($\mu\text{g.}$)	$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (acetone)	Amount/insect ($\mu\text{g.}$)
First	250	6	0.018	0.13	0.028	0.21
Second	105	4	0.023	0.38	0.052	0.97
Third	63	3	0.015	0.40	0.037	1.4
Fourth	34	3	0.028	2.1	0.021	1.8
Fifth	32	5	0.018	3.0	0.026	5.5
Immature (male)	19	7	0.029	8.4	0.0078	3.5
Immature (female)	27	7	0.027	10.7	0.0061	3.0
Mature (male)	35	9	0.029	15.4	0.0050	3.4
Mature (female)	30	9	0.036	35.8	0.0045	5.4
Immature (female) (blue)	4	4	0.0061	1.8	0.413	15.5
Mature (male) with protozoan infection	4	2	0.015	9.1	0.0077	5.8
Mature (female) with protozoan infection	7	6	0.0072	7.4	0.011	15.0

Table 2. Carotenoid distribution in solitary *Locusta*

Stage	No. of insects examined	No. of batches examined	β -Carotene		Astaxanthin	
			$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (petrol)	Amount/insect ($\mu\text{g.}$)	$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (acetone)	Amount/insect ($\mu\text{g.}$)
First	46	2	—	—	0.029	0.22
Fourth	4	1	—	—	0.044	2.9
Fifth	7	2	—	—	0.030	5.0
Immature (male and female)	4	4	0.039	11.4	0.016	5.8
Mature (male)	3	3	0.035	19.1	0.010	7.1
Mature (female)	2	2	0.026	12.5	0.021	12.6

Table 3. Carotenoid distribution in gregarious *Schistocerca*

Stage	No. of insects examined	No. of batches examined	β -Carotene		Astaxanthin	
			$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (petrol)	Amount/insect ($\mu\text{g.}$)	$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (acetone)	Amount/insect ($\mu\text{g.}$)
First	56	2	0.001	0.026	0.044	0.13
Second	36	3	0.031	0.63	0.34	0.72
Third	44	4	0.035	1.7	0.026	1.6
Fourth	22	3	0.016	1.7	0.028	4.1
Fifth	5	2	0.026	8.7	0.024	7.6
Immature (male)	12	5	0.038	22.9	0.016	12.5
Immature (female)	11	5	0.028	13.6	0.013	8.3
Mature (male)	6	3	0.069	44.0	0.0043	3.5
Mature (female)	2	1	0.061	55.8	0.014	16.5

Table 4. Carotenoid distribution in solitary *Schistocerca*

Stage	No. of insects examined	No. of batches examined	β -Carotene		Astaxanthin	
			$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (petrol)	Amount/insect ($\mu\text{g.}$)	$E_{1\text{ cm.}}^{1\%}$ 450 m μ . (acetone)	Amount/insect ($\mu\text{g.}$)
First	42	1	—	—	0.039	0.39
Fourth	3	1	0.042	5.5	0.053	5.6
Fifth	3	1	0.046	26.4	0.023	17.7
Immature (male and female)	5	3	0.045	25.3	0.014	10.1
Mature (male and female)	3	2	0.064	47.5	0.017	16.2

Schistocerca there is no difference in respect of carotenoid distribution between the solitary and gregarious phases; further, there is no difference between *Locusta* and *Schistocerca* in the concentrations of the pigments; but there is a tendency for adult *Schistocerca* to contain more of the pigments

Fig. 1. The changes in the astaxanthin concentration ($E_{1\text{ cm}}^{1\%} 450 \text{ m}\mu$) of both phases of *Locusta* and *Schistocerca* during development. The vertical lines indicate the standard deviation of the results.

Fig. 2. The changes in the carotene concentration ($E_{1\text{ cm}}^{1\%} 450 \text{ m}\mu$) of both phases of *Locusta* and *Schistocerca* during development. The vertical lines indicate the standard deviation of the results.

than do adult *Locusta*; this is again merely a reflexion of the weight variations between the species. There is, however, a marked and important difference in the storage of the two pigments during development. As this difference obtains in both species, irrespective of phase, 'grand means' of the concentrations (measured as $E_{1\text{ cm}}^{1\%}$) and amounts of the two pigments have been calculated for each stage and plotted in Figs. 1-3.

Considering concentrations first, it will be seen (Fig. 1) that the astaxanthin concentration remains reasonably constant from birth to the third hopper stage, and thereafter diminishes until in mature

adults it is not more than one-quarter to one-third of that of the early hopper values. With carotene the situation is different (Fig. 2); the carotene concentration at birth increases somewhat as soon as the insects begin to eat and then remains near a constant value until the insects become adult when the concentration increases until at maturity it is at least twice that at birth.

This difference between carotene and astaxanthin is reflected in the amount of the pigments present in the developing insects (Fig. 3). During the hopper stages the astaxanthin content increases gradually but rather slowly; the increase ceases at adult

Fig. 3. The changes in the carotenoid content of locusts during post-embryonic development: A, astaxanthin content; B, β -carotene content.

emergence, and throughout adult life the levels remain constant at or just below the fifth-stage levels. The increase in β -carotene content in general parallels that of astaxanthin until the insects become adult when, in contrast to astaxanthin, it increases during adult life; this is especially marked after sexual maturity when considerable stores are found in the fatty tissues.

Carotenoids in the eggs. Newly laid eggs of both *Schistocerca* (Chauvin, 1941b; Goodwin & Srisukh, 1948, 1949) and *Locusta* (Goodwin & Srisukh, 1948, 1949) contain β -carotene only. In Fig. 4 are recorded the results of an investigation into the quantitative and qualitative changes in the carotenoid content of the developing *Locusta* egg. It will be seen that apparently β -carotene takes no part in the process of embryonic development until near the end of the period. It is only on about the 7th day that it begins to disappear and astaxanthin to appear; both these processes then continue until the insect is hatched. A similar experiment using *Schistocerca* eggs yielded very similar results, and is not recorded here.

It will be noted that the average carotene content of a *Locusta* egg is $0.38 \mu\text{g}$. This means that an average egg pod of 50 eggs will contain about $20 \mu\text{g}$ of β -carotene; the corresponding mean carotene level in *Schistocerca* eggs is about $1.0 \mu\text{g/egg}$.

Carotenoids in exuviae. The appearance of carotenoids in the exuviae of any hopper stage has never been detected.

Carotenoids in dark blue Locusta. During the course of this investigation four atypical immature females were examined; they were very dark blue, had a metallic lustre, and were progeny of normal parents. However, it was noted that the subcutaneous fatty material was completely colourless, although one would have expected it to have been bright yellow owing to the presence of stored β -carotene. Chromatography of the carotenoid extract showed that the carotenoid present was almost completely astaxanthin; β -carotene was detected, but only in traces (see Table 1). The astaxanthin levels were much higher than normal.

Fig. 4. The changes in the carotenoid content of fertilized *Locusta* eggs during embryonic development: A, astaxanthin content; B, β -carotene content.

Carotenoids in infected insects. It has been possible to examine locusts infected with a protozoan, probably *Phistophora*. The infected insects have a normal external appearance except that the outside of the abdomen becomes a characteristic pink. When they were opened it was found that the fatty tissues had lost their yellow colour and, except for the occurrence of pink spots, were almost white; it was therefore no surprise to find that, compared with healthy insects, the carotene contents of both males and females were very low; this was most marked in the case of heavily infected insects, especially females. On the other hand, the astaxanthin contents of both sexes were normal (Table 1). It was noted that the developing eggs of infected females still contained carotene, but it was not possible to ascertain the amount.

DISCUSSION

Chauvin (1941b) stated that the pink carotenoid recently identified as astaxanthin (Goodwin & Srisukh, 1948, 1949) is characteristic of immature locusts and that the yellow carotenoid (β -carotene)

is characteristic of adults. This generalization can now be seen to have only a limited validity, but it did suggest the existence of an important difference between the metabolism of β -carotene and astaxanthin in hoppers and adult locusts. This difference has now been quantitatively demonstrated. At birth about 70 % of the total carotenoids is astaxanthin; this value gradually decreases until the fifth hopper stage; however, when the locusts become adult the accumulation of β -carotene proceeds at an enhanced rate whilst the amount of astaxanthin present remains stationary. This results in astaxanthin being never more, and often much less, than 30 % of the total carotenoids in adult insects.

It is not known why astaxanthin storage ceases when locusts become adult. It may be due to the fact that the rate of synthesis is reduced or stopped altogether, or that synthesis continues at the same rate, but that adult locusts utilize (or destroy) the pigment to a greater extent than do hoppers. It is interesting to note that wings, which are fully formed only in adults, contain relatively more astaxanthin than carotene; however, as they contain relatively little pigment compared with the rest of the body (Goodwin & Srisukh, 1949) it is not possible to postulate that astaxanthin synthesis in adults is diverted to the wings rather than to the body.

Formation of astaxanthin. This pigment, which is not present in the food of the locust, must be produced in one of three ways: (a) *de novo*, (b) by oxidation of alimentary carotenes, or (c) by oxidation of alimentary xanthophylls. The first possibility is almost certainly ruled out, for a survey of carotenoid biochemistry reveals no authenticated instance of carotenoid synthesis in animals (Goodwin, 1949a). Up to the present it has not been possible to decide which (if not both) of the other alternatives operates, for, as no adequate artificial diet is yet available, it has not been possible to carry out feeding experiments. No plant xanthophylls are ever found in locusts outside the alimentary tract, so that these pigments might either be converted quantitatively into astaxanthin or be quantitatively excreted; the latter possibility was put to experimental test. The relative amounts of carotenoids and xanthophylls were determined in *Locusta* faeces collected at all stages of development, together with the grass upon which they were feeding. It was considered that if only xanthophylls were found in the faeces or if the faecal xanthophyll/carotene ratio was very much higher than that of grass then it would be strong presumptive evidence that xanthophylls were preferentially excreted. However, it was found that locust faeces always contained a considerable amount of undigested or partly digested grass and were thus always very rich in both xanthophylls and carotenes, which occurred in ratios not differing significantly from those of the ingested grass. It was

therefore impossible to reach any conclusions as to the ability of the locust to absorb xanthophylls.

There is one fact which favours the suggestion that carotene is the precursor of astaxanthin. Astaxanthin is formed during the incubation of the egg, and, as it is unlikely to be produced *de novo*, carotene appears to be the only possible precursor.

Carotenoids in the developing egg. It has been shown quite clearly that β -carotene is used up during the later stages of embryonic development and that astaxanthin is formed. As has just been suggested, it is likely but not certain that astaxanthin is formed by the oxidation of β -carotene; even if this were so the astaxanthin produced does not account for all the β -carotene which disappears. What function the β -carotene plays in the developing locust egg is not known. Although carotenoids are often preferentially stored in the eggs of lower animals, little is known of their function, and it appears that the present work is the first to demonstrate that β -carotene is actively metabolized during embryonic development of the egg. In contrast, it should be noted that Mann (1946) and Suomalainen (1939) have reported no loss of carotenoids during the development of hens' eggs.

Any function in locust eggs which may eventually be assigned to carotenoids is perhaps unlikely to be a general one, for a number of insects, amongst which is the orthopteran *Blatella germanica* (McCay, 1938; Bowers & McCay, 1940), can exist on a diet free from carotene or vitamin A.

As has been stated earlier, the amount of carotene in a *Locusta* egg pod, containing, on the average, 50 eggs, can amount to at least 20 μg . This is of the same order as the amount found in a female without eggs and in an adult male. It seems, then, that females absorb more carotene, perhaps by eating more food, to provide the required amount for their eggs, for these obviously do not obtain their carotene at the expense of the other body tissues. The results of a limited number of experiments indicate that *Locusta* and *Schistocerca* eggs contain respectively about 0.4 and 1.0 μg . of β -carotene/egg. This difference is due mainly to the difference in size, for the weight of a *Schistocerca* egg is about 0.01 g. whilst that of a *Locusta* egg is about 0.06 g. The concentration of carotene in *Locusta* and *Schistocerca* eggs, therefore, does not differ significantly, and is about 100 μg ./g. of eggs (wet weight); this is of the same order but somewhat higher than the value for *Schistocerca* eggs reported by Brodskis (1944) and Brodskis & Rungs (1944) who obtained 95 μg ./g. dry weight; assuming a value of 30 % for the dry weight of the eggs, this reduces to about 30 μg ./g. wet weight. On the other hand, the carotene content of grasshopper (*Melanoplus bivattus* Say) eggs has been reported to be 246 μg ./g. dry weight (Grayson, 1942; Grayson & Tauber, 1943).

Relationship between carotenoids and colour of locusts. It has already been stated that there are no significant differences between the carotenoid contents of gregarious and solitary locusts, but it will be interesting to consider the other important stage of colour differentiation; that accompanying the onset of sexual maturity. At this stage males become yellow and females dark brown. It has been established in the case of *Locusta*, at least, that acridioxanthin, the main pigment of locusts other than the carotenoids, is unchanged in amount, state of oxidation and position in the integument* (Goodwin, 1949b) in males and females. The experiments reported here (see Table 1) have shown that the concentration of carotene in both sexes is the same; the amounts in the females are somewhat greater owing to their greater size. It seems reasonable to assume that the yellow colour which the males assume when they reach maturity is due to a migration of part of their carotenoids to the outer layers of the cuticle, for acetone will dissolve out a considerable amount of carotene from a piece of yellow cuticle carefully freed from fat. Owing to technical difficulties histological confirmation of this has not yet been achieved, but it is interesting to note in this respect that in males infected with *Plistophora* (Table 1) the loss of carotene is less than in infected females. This could be explained by assuming that the cuticle carotene in the males was only slightly affected compared with that stored in the adipose tissues; a similar survival of 'functional' carotene was noted in the eggs of infected females. Chauvin (1941b) has previously claimed that the yellow coloration of mature *Schistocerca* is due to β -carotene.

Although the striking colour differences between solitary and gregarious phases cannot be due to differences in the carotenoid content of the insects as a whole, it is very likely that they are due to differences in carotenoid distribution especially near the cuticle.

Carotenoid distribution in blue locusts. In the atypical immature female *Locusta* the acridioxanthin content was normal (Goodwin, 1949b), but instead of containing a mixture of carotene and astaxanthin, the former preponderating, only traces of carotene were present, but astaxanthin was present in excess (Table 1); further, astaxanthin must have been present in the integument, for the fatty tissues of the body were colourless. It may be that the outstanding blue colour of these locusts was due to an integumentary astaxanthin-protein, such as occurs in the purple patches of lobster carapaces (Kuhn & Lederer, 1933). Such a (brown) astaxanthin complex has been shown to occur in the wings of normal locusts, but up to now has not been found in their

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integuments (Goodwin & Srisukh, 1949). It is possible that these insects were mutants in which an enzyme was produced (or activated) which either converts β -carotene very efficiently into astaxanthin or oxidatively destroys it. Such genetic control of carotenoid metabolism in insects has been noted previously (Gerould, 1921; Uda, 1919; Przibram & Lederer, 1933).

The value of locusts as a nutritional source of carotene. The nutritional importance of locusts, especially in North Africa, cannot be overlooked (Grant, 1948); as the carotene content of a gutted, mature locust is at least 30 $\mu\text{g.}$, the value for an intact animal will be higher owing to the presence of carotene in the alimentary tract. Our investigations on whole *Locusta* indicate that their carotene content varies from 35 $\mu\text{g.}$ for mature males to 70 $\mu\text{g.}$ for mature females with eggs. An average value for mature *Schistocerca* has been given (Brodskis & Rungs, 1944) as 256 $\mu\text{g./g.}$ dry weight which, assuming 70 % of water, reduces to 80 $\mu\text{g./g.}$ wet weight and to about 120 $\mu\text{g./insect.}$ It will be seen that locusts are as good a source of β -carotene as are most green vegetables. In fact, it is quite possible that humans would utilize 'locust carotene' rather better than 'plant carotene', for the locust pigment is associated with considerable amounts of fat and it is well known that the presence of fat generally improves carotene absorption (see e.g. Hume & Krebs, 1949). It is interesting to note that from the carotene viewpoint the higher price charged for well-developed females (Dubois, 1893) is justified.

SUMMARY

1. The distribution of β -carotene and astaxanthin has been studied in the solitary and gregarious phases of two locust species: *Locusta migratoria migratorioides* R. & F. and *Schistocerca gregaria*

Forsk. The locusts were examined at most stages of development, and the relative distribution in body, head and pronotum, and legs was also studied.

2. In both species no significant differences in the carotenoid distribution in the three main body regions occur between solitary and gregarious insects; further, no differences were noted between the two species.

3. The relative amounts of astaxanthin and carotene alter during development. In the early hopper stages astaxanthin comprises about 70 % of the total carotenoids, whilst in mature insects this drops to below 30 %.

4. When the β -carotene content of the eggs is allowed for, no differences are found in the carotenoid concentration of male and female insects.

5. No carotenoids were detected in locust exuviae.

6. Newly laid *Locusta* and *Schistocerca* eggs contain β -carotene to the extent of about 100 $\mu\text{g./g.}$ (wet weight). During the later stages of pre-embryonic development β -carotene disappears and astaxanthin is formed.

6. In four atypical *Locusta*, which were very dark blue, astaxanthin occurred in considerably increased amount and carotene only in traces.

7. *Locusta* infected with the protozoan *Plistophora* store very little carotene but normal amounts of astaxanthin.

8. The possible importance of locusts, which contain β -carotene (provitamin A) in amounts similar to those found in green vegetables, as a source of vitamin A in some native dietaries is indicated.

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The Enzymic Degradation of Adenosinetriphosphate

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The present investigation arose from the observation that samples of adenosinetriphosphate (ATP), freshly prepared from skeletal muscle, gave a satisfactory phosphate cleavage with myosin adenosinetriphosphatase, whilst samples stored as the barium salt at room temperatures were very deficient in this respect. By the use of a specific inorganic pyrophosphatase (Bailey & Webb, 1944) it was later shown (Bailey & Webb, unpublished) that such specimens contained as much as 12% of the labile phosphorus as inorganic pyrophosphate, indicating a breakdown of ATP to this latter substance and adenylic acid. It may be recalled that Lohmann (1928), before his characterization of ATP, considered the labile phosphorus of muscle to be inorganic pyrophosphate, and did in fact isolate the crystalline sodium salt. Later, he considered it to have arisen during the preparative procedure (Lohmann, 1931).

It is clear that ATP preparations decomposing in this way may show a satisfactory ratio of total phosphorus/labile phosphorus/total nitrogen, and other criteria of purity must be employed. For this purpose enzyme methods have been explored. The purity both of laboratory and commercial specimens of ATP has been assessed, and changes occurring during known conditions of storage have been followed.

EXPERIMENTAL

Enzyme preparations

Myosin adenosinetriphosphatase. Myosin was prepared in the conventional manner from rabbit muscle (see Bailey, 1942), and was five times precipitated from water. It was stored in 0.5M-KCl at pH 7 and 0° in presence of a trace of toluene, and was efficient enzymically over a period of 3-4 weeks.

Myokinase. The method of preparation followed strictly that of Colowick & Kalckar (1943). The enzyme was stored

at 0° as an ammonium sulphate filter cake, and dissolved in dilute veronal buffer (pH 7) before use. As a paste, the enzyme remains active indefinitely.

Yeast inorganic pyrophosphatase. The purified enzyme prepared by Bailey & Webb (1944) was found to be active after storing at 0° for 5 years. In the initial experiments, a new batch of crude enzyme was prepared from brewer's yeast. It was found that yeast autolysate, after dialysis, contained not only the very active pyrophosphatase, but also a weak, Mg-activated adenosinetriphosphatase. After submitting the autolysate to the first four stages of the fractionation procedure of Bailey & Webb (1944), this latter enzyme had disappeared, leaving a very active pyrophosphatase suitable for the present work. Although the original enzyme has been used throughout, the preparation of new enzyme does not require the laborious procedure first employed. Preparations must be stored as an ammonium sulphate-protein paste at 0°.

Potato apyrase. This enzyme, discovered by Berger, Colowick & Slein (unpublished; see Kalckar, 1944a), splits off two phosphate groups from ATP. In critical experiments, however, it was found that preparations made according to the directions of Kalckar (1944a) are feebly active with respect both to inorganic pyrophosphate and adenylic acid. Our findings are supported in a recent paper by Krishnan (1949), who has compared the substrate specificity of crude and purified enzyme. In high concentration, even the purified enzyme possesses nucleotidase activity, but not at concentrations which are still able to split off two phosphate groups from ATP. It would appear, therefore, that potato apyrase is mixed with other phosphatases, and the usefulness of this enzyme is somewhat limited.

Incubation conditions

General. After establishing the complete specificity of the enzyme preparations, three test systems were adopted: the splitting of ATP (a) by myosin adenosinetriphosphatase, (b) by myosin adenosinetriphosphatase in presence of myokinase, and (c) by yeast inorganic pyrophosphatase. Additional experiments with potato apyrase were sometimes carried out. In (a), only the terminal phosphate group is split, whilst in presence of myokinase the adenosinedi-