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Carotenoid Distribution in the Gonads of the Limpets *Patella vulgata* and *Patella depressa*

By T. W. GOODWIN

Department of Biochemistry, The University of Liverpool

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Goodwin & Taha (1950) have shown that the four carotenoids, β -carotene, echininone, cryptoxanthin and zeaxanthin, occur in the male and female gonads of both *Patella vulgata* and *P. depressa*. The present report is concerned with the quantitative aspects of the distributions of these carotenoids as they are affected by sex, species and environment.

EXPERIMENTAL

P. vulgata, from the same region of the shore and of approximately equal size, were collected from Port St Mary, Isle of Man. They were delivered alive to the author. Samples from other sources, namely Plymouth, Trevone (Cornwall), Cullercoats (Northumberland), and Aberdeen were also occasionally obtained. Samples of *P. depressa* were obtained only from Plymouth.

The gonadal carotenoids were extracted and separated chromatographically by the previously described method (Goodwin & Taha, 1950). The various fractions were dissolved in a known volume of light petroleum (b.p. 40–60°), and the amount of each pigment present was determined spectroscopically using a Beckman spectrophotometer (see Goodwin, 1949). When the pigments were not separated chromatographically the total pigment concentration was determined by measuring the extinction of the test solution at 450 m μ . and basing the subsequent calculation on the assumption that all the colour was due to β -carotene. This is an approximation leading only to a very small error (about 5% at most) which, in any case, is constant throughout the series.

RESULTS AND DISCUSSION

A considerable mass of data has been condensed and collected in Tables 1–4.

Relative amounts of carotenoids in male and female gonads. The results recorded in Table 1 clearly indicate that no significant differences are observed between the relative distribution of the four component carotenoids in male and female gonads; especially interesting is the fact that no differences occur in this direction between the olive-green and the brown types of ovaries. Comment must be made on the very high standard deviation for the values of echininone and cryptoxanthin, especially in ovaries; this is undoubtedly due to the technical difficulties in effecting a complete separation of these two pigments in ovary extracts owing to the presence of large amounts of steroids which interfere with the normal development of the chromatogram.

The amount of pigments in male and female gonads. In Table 2 are collected the data obtained for the gonads of limpets of approximately the same age (size) collected from the same shore region at Port St Mary over a period of a month. Calculations according to the 't' test indicate that the concentration in the brown ovaries is significantly higher ($P < 0.01$) than is the concentration in green ovaries, but that there is no difference in the amount of carotenoids present in the two coloured types of ovaries; brown ovaries are therefore significantly smaller than the green ovaries. It is also to be noted that the brown samples varied between themselves much more than did the green samples.

When male and female gonads are compared it is found that their concentrations are not significantly different, but that the amount per limpet is greater in the male than in the female; this reflects the fact

Table 1. *The distribution of the constituent carotenoids in the male and female gonads of limpets*

Material	No. of batches examined	Amount of pigment expressed as percentage of total							
		β -Carotene		Echininone		Cryptoxanthin		Zeaxanthin	
		(%)	(s.d.)	(%)	(s.d.)	(%)	(s.d.)	(%)	(s.d.)
<i>Patella vulgata</i>									
Green	11	45.2	17.6	18.9	13.4	19.0	16.9	16.9	7.1
Brown	4	44.3	—	19.8	—	23.7	—	22.2	—
Pink	9	51.9	11.9	16.7	6.9	15.3	3.6	16.1	6.8
Mean		47.5		18.2		18.5		15.8	
<i>P. depressa</i>									
Orange	1	43.4	—	24.9	—	—	31.7	—	—

Table 2. *Total carotenoid distribution in male and female gonads of Patella vulgata collected from Port St Mary*(All samples collected between 18 October and 20 November 1947. Results expressed as mean $\pm$ s.d.)

Material	No. of batches examined	Total carotenoid concentration (μ g./g.)	Total carotenoid content per limpet (μ g.)
Female gonads			
Brown	14	92 $\pm$ 51	69 $\pm$ 43
Green	14	49 $\pm$ 15	64 $\pm$ 25
Male gonads	24	68 $\pm$ 38	106 $\pm$ 37

Table 3. *Carotenoid content of gonads of Patella vulgata collected from different regions of the British Isles*

(All samples collected between 18 October and 18 November 1947.)

Material and source	No. of batches examined	Carotenoid concentration (μ g./g.)		Total carotenoid content per limpet (μ g.)	
		Brown	Green	Brown	Green
Female gonads					
Plymouth	6	37	29	51	37
Trevone	3	12	11	11	13
Cullercoats	3	52	—	40	—
Aberdeen	3	41	40	42	32
Male gonads					
Plymouth	9	47	—	55	—
Cullercoats	3	89	—	24	—
Aberdeen	9	43	—	41	—

Table 4. *Total carotenoid concentration in gonads of Patella depressa*

(Material collected at Plymouth between 22 and 30 October 1947.)

Material	No. of batches	Total carotenoid concentration (μ g./g. fresh wt.)	Total carotenoid content per limpet (μ g.)
Ovaries (brown)	3	10	25
Ovaries (green)	3	6.5	24
Testes	6	4.1	89

that at the time specimens were taken the testes were larger than were the ovaries. A different situation might arise at other seasons of the year.

Carotenoid content of limpets collected from various regions in the British Isles. A number of samples

from various regions became available during the autumn of 1947 and their examination led to the results recorded in Table 3. It will be noted that the results are of the same order as those obtained for limpets from Port St Mary. Little more than that can be said because, in general, these limpets were *ad hoc* collections from different localities of the same region and of different sizes, whilst those obtained from Port St Mary were always strictly comparable. It should be pointed out that differences in the ovaries, which are more marked than in the testes, are probably due to the different stage of spawning reached in the various regions. An extended investigation on the effect of spawning on the carotenoid content of both testes and gonads is in progress and, although at the moment incomplete, it is obvious that ovaries are more affected than are the testes.

Carotenoid content of Patella depressa. A few samples of *P. depressa* were obtained from Plymouth at the same times as were *P. vulgata*. It has already been shown that the carotenoid constituents are the same in *P. depressa* and *P. vulgata* (Goodwin & Taha, 1950) and the present data (recorded in Table 4) indicate that the quantitative distribution of the pigment is also similar in the two species. This is specially marked in the case of the testes, the rather greater differences between the ovaries of the two species again being probably due to the different stages of spawning in the samples examined. One of the most interesting problems concerning limpet ovaries is the question of the production of brown and green ovaries. It has already been shown (Goodwin & Taha, 1950) that carotenoids play no major part in the production of green ovaries, a chromoprotein containing a non-carotenoid prosthetic group being the most important factor. The increased concentration of carotenoids in brown ovaries is unlikely to cause the change from green to brown; more probably it depends on the observation that the isolated green chromoprotein turns brown on addition of a few drops of dilute acetic acid. The brown and green colours may well be due, therefore, to variations in pH of the ovaries.

SUMMARY

1. The four carotenoids, β -carotene, echininone, cryptoxanthin and zeaxanthin, occur in the gonads of *Patella vulgata* in the ratios 5:2:2:2.

2. There are no significant deviations from this distribution in either the brown or green ovaries or in the testes.

3. A similar distribution of the same pigments is found in *Patella depressa*.

4. Although brown and green ovaries contain the same amounts of carotenoids, the brown have a higher concentration, indicating that brown gonads are generally smaller than the green.

5. The concentrations in the testes and ovaries are very similar, but the content of the testes is greater; this, again, merely reflects the weight relationship of ovaries and testes at the time of sampling.

6. Limpets collected at the same time from different regions of the British Isles contain comparable amounts of carotenoids.

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The Reaction of Oxidizing Agents with Wool

2. THE SORPTION OF HYDROGEN PEROXIDE

BY P. ALEXANDER, D. CARTER AND C. EARLAND
Research Department, Wolsey Ltd., Leicester, England

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Hydrogen peroxide was shown by Harris & Smith (1936*a, b*, 1937*a, b*) to oxidize the cystine in wool partly to cysteic acid and partly to cystine disulphoxide. Rutherford & Harris (1938) found that the reaction was far from specific, and that many other groups besides the disulphide bond were oxidized, and some evidence for preferential attack on the free amino groups of the basic amino-acids was found.

Elöd & Zahn (1940) showed that silk, which contains no cystine, dissolved completely in 3% (w/v) hydrogen peroxide (pH 4) after 3 days at 60°. Wool (Elöd & Zahn, 1942) behaved likewise, and the solution obtained contained no high molecular weight fractions. Hydrogen peroxide is therefore undoubtedly capable of breaking peptide bonds, although in view of the stability of polyglycine to

this reagent it seems probable that the attack is preceded by the oxidation of a reactive side group. The rate of reaction of wool with hydrogen peroxide is slow and at room temperature no oxidation can be detected, except in very alkaline solutions for several hours. All the reactions discussed were carried out at elevated temperatures.

While studying bleaching it was found that there was a small but detectable decrease in the peroxide concentration immediately on immersing the wool in the solution, after which no further change could be detected for several hours. A similar effect was observed with nylon and silk but not with cotton. The peroxide, which very rapidly disappeared, was unambiguously shown to be physically held by the nitrogen-containing fibres and not to have reacted