Carotenoids in an Acanthocephalid Worm *Pallisentis nagpurensis*

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Several species of acanthocephalid worms frequently show red, orange, yellow or brown colours. However, relatively little is known about their chemical basis and host parasite reltionships. Histochemic l studies carried out by Van Cleave and Rausch (1950) and Nadakal (1963) showed the occurrence of carotenoids in *Arhythmorhynchus comptus*, and *Pallisentis* sp. respectively. However, as the previous work on carotenoids of acanthocephalid worms was based purely on histochemical methods, which permit only their broad characterization, it was thought desirable to study them with physico-chemical methods. The present paper, therefore, deals with the results of a study on the carotenoid pigments of *Pallisentis nagpurensis* (=*Farzandia nagpurensis*, Bhalerao, 1932) involving extraction, chromatographic separation and spectrophotometric absorption analysis.

Materials and Methods

Specimens of the worms used in this study were collected from the gut of fresh water fish, *Ophiocephalus striatus*. Nearly 2,000 fishes were dissected from time to time and the worms, measuring on an average 12 mm ×0.6 mm, were recovered, washed in saline, put in small tightly corked glass tubes and kept under refrigeration. When sufficient quantity of parasite material was thus accumulated, it was dried in a desiccator and then homogenized. For extraction and analysis of the pigments, the methods outlined by Fox and Pantin (1941) were followed. For chromatographic separation of the pigments, adsorption columns were prepared in cylindrical glass tubes measuring 20 cm×8 mm. Setting up of the adsorption column, development of the chromatogram and separation of coloured zones were carried out as described by Karrer and Jucker (1950). Activated alumina appeared the best adsorbent for carotenoids and petroleum ether the best developing solvent for the epiphasic pigments. Partition tests were carried out with a tiny separatory funnel designed by the authors. Pigment fractions separated by chromatography, were eluted in petroleum ether with a few drops of 90% methanol. Identification of the pigment was made by colour reaction, solubility in organic and inorganic solvents, partition tests, fluorescence, adsorption behaviour and spectrophotometric absorption analysis using a Spectronic 20 (Bausch and Lomb). The pigments of the coloured gut contents of the host fish were also subjected to the same treatments and observation, for identification. Recorded measurements for wave lengths are expressed in millimicrons.

Observations

It was found that all the worms recovered from the intestine of the fish were not coloured. Some of them were orange, red, brown, or yellow, while others colourless. Colouration of the worms appear to depend upon the nature of the gut contents of the host. Worms recovered from the colourless gut contents were invariably colourless ; but the converse was not true of the worms found in the coloured gut contents. Worms also showed variations in the intensity of colouration regardless of their age or size.

Remains of plant materials and crustaceans formed the major components of the gut contents. Microscopic examination of the live specimens and their teased body tissues indicated that the pigment granules are more or less evenly distributed in the body wall. *Preliminary tests:* Using desiccator-dried parasite materials, the solubility of the pigments was tested. It was found that the pigments dissolved in organic solvents such as acetone, methanol, ethanol, xylol and chloroform. They were insoluble in water, 10% formalin, weak acids and alkalies. Chloroform solution of pigments on mixing with concentrated sulphuric acid gave greenish blue colour reaction. Petroleum ether extract of the pigment placed on a piece of Whatman No. 1 filter paper and exposed to ultraviolet rays showed green fluorescence. The pigment extract in petroleum ether on exposure to atmospheric air showed a tendency to lose colouration and this was probably due to autoxidation. In partition tests the pigments behaved invariably as epiphasic.

These observations and tests were also performed for the pigments of the gut contents and the same results were obtained. *Chromatographic adsorption behaviour:* Three coloured bands developed on the adsorption column in the case of pigment extract from worms. They were: (1) a faint narrow yellow band at the top of the column (2) a slowly moving thick orange band and (3) a yellow band moving fast to the bottom of the column (Fig. 1). The narrow yellow band that remained at the top of the column faded out during the course of its downward movement.

The pigments of the gut content extract also behaved chromatographically in the same manner as did the parasite pigments. However, the bands were rather lesspro nounced. *Spectral properties:* The pigment of the lower yellow band eluted with petroleum ether showed two absorption maxima at 421 and 442 and that of the orange band two absorption maxima at 425 and 452 (Fig. 2). The absorption spectra of the pigments chromatographically separated from the extracts of gut contents were not sharply defined.

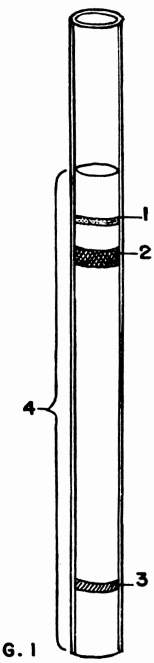

FIG. I

Fig. 1. Pigment bands on the adsorption column.
1. Pale yellow band
2. Orange band
3. Yellow band
4. Alumina column

In an attempt to study whether or not the worms are capable of readily absorbing the carotenoid pigments through their body wall the following simple experiment was conducted:

Carotenoid pigments were extracted from desiccator-dried carrot using petroleum ether and allowed to dry. The pigment was then added to the oily gut contents freshly collected from the host fish and diluted with saline. 10 colourless worms, washed thoroughly in saline, were then placed in this mixture and kept at the room temperature. After about three hours, the worms were taken out, rinsed well in saline and examined under the microscope. It was found that the worms had absorbed the pigments and appeared deeply coloured.

Discussion

The results of the preliminary tests and

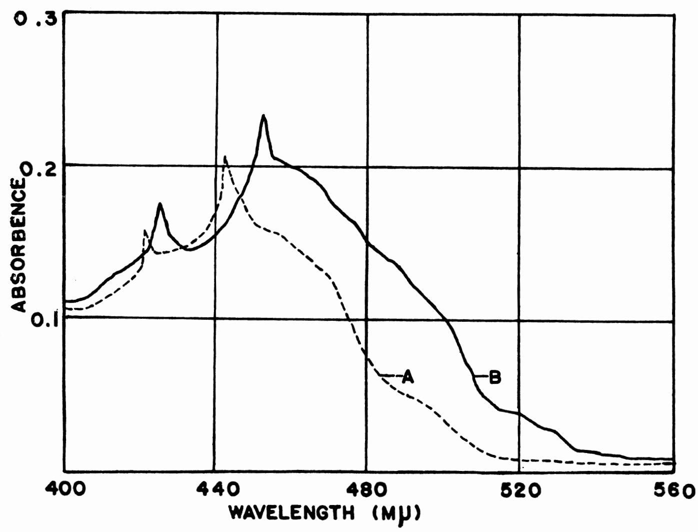

FIG.2

Fig. 2. Spectral curves of the pigments.
A. Yellow band-oxide of α-carotene
B. Orange band-oxide of β-carotene

observations suggested that the pigments found in *Pallisentis nagpurensis* are carotenoids and this finding is of course in conformity with the results obtained by Nadakal (1963). The absorption maxima of the pigments in petroleum ether of the orange band developed on the adsorption column at 425 and 452 suggeted that it may be an oxide of β-carotene, probably mutatochrome; whereas the absorption maxima at 421 and 442 of the pigment represented by the yellow band, an oxide of α-carotene resembling flavochrome. The epiphasic behaviour of pigments in partition tests and chromatographic behaviour may be adduced as additional evidence for this conclusion. According to Karrer and Jucker (1950) mutatochrome in petroleum ether shows two absorption maxima at 427 and 456 and flavochrome two maxima at 422 and 450. They have been recorded both as natural pigments and as products formed by oxidative changes of β-carotene and α-carotene respectively. Both are epiphasic in partition tests. In the present instance, the time and site of oxidation of the pigments are obscure. It is likely that it may occur in the gut of host fish before their absorption by the worms. The possibility of oxidative changes occurring in the pigments during experimental procedures cannot also be ruled out.

It was remarkable that in partition tests the hypophasic part of the parasite extract contained no carotenoids and that all the pigments remained epiphasic.

The pigments extracted from the gut contents of the host fish also behaved in the same way in partition tests and on the adsorption column as did the parasite pigments. There was clear separation of three bands, the top one being unstable. However, spectrophotometric absorption analysis could not be effectively carried out because of insufficiency of the pigments on elution. Epiphasic and chromatographic behaviour of pigments seem to favour the suggestion that the gut content extract also contain the same oxides of β-carotene and α-carotene.

Concerning the host parasite relationship of the pigments, it appears that the parasites absorb the pigments as such from the intestine of the host. The absorption of the carotenoid through the body wall of the worm is suggested by the experiment with carrot pigments and live worms. Lee(1966) has recently reported that absorption of food materials in acanthocephalid worms takes place through the pores and canals in the cuticle and striped layer of the body wall rather than through the general body surface. Nutritional aspect of pigmentation in free living as well as parasitic animals has been well established by several workers like Fox and Pantin (1941); Llewellyn (1954) and Nadakal (1960 a, 1960 b). The source of pigments for the worms can thus be traced to the gut contents of the host, the major bulk of which is represented by remains of plants and crustaceans. Plants and crustaceans are known to be rich in carotenoid pigments such as α-carotene, β-carotene and astaxanthin, and the available literature on this subject has been reviewed by Fox (1953) and Goodwin (1954). The parasites may obtain their pigment supply from food materials metabolically conditioned in the gut of the host fish. The oily nature of the intestinal contents of the fish must certainly facilitate the pigment uptake by the worms. Biologically the carotenoid pigments found in these worms may be of negligible importance. They may be indicators of the degree of unsaturation of parasite fat with an incidental partition coefficient favouring accumulation as in certain larval trematodes studied by Nadakal (1960 a).

Summary

The carotenoid pigments extracted from the acanthocephalid worm, *Pallisentis nagpurensis,* resolved into two bands on the adsorption column of alumina, one orange and the other yellow. The absorption maxima of the pigment in the orange band in petroleum ether at 425 mμ and 452 mμ indicate that it is an oxide of β-carotene, probably mutatochrome, while those of the pigment in the yellow band at 421 mμ and 442 mμ, an oxide of α-carotene, resembling flavochrome. Besides preliminary tests, epiphasic and chromatographic behaviour suggested that the coloured gut contents of the host fish *Ophiocephalus striatus* also contained these pigments. Available evidence points to the view that the parasites absorb these pigments from the gut of the host fish and store them as such in their body wall tissue.

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References

1) Fox, D. L. (1953): Animal Biochromes and structural colours, (Cambridge University Press).
2) Fox, D. L. and Pantin, C. F. A. (1941): The colours of the plumose anemone, *Metridium senile*. Philos. Trans. B, 230, 415–450.
3) Goodwin, T. W. (1954): Carotenoids, their comparative biochemistry (Chemical Publishing Company, New York).
4) Karrer, P. and Jucker, E. (1950): Carotenoids (Elsevier Publishing Co. Inc., New York).
5) Lee, D. L. (1966): The structure and composition of helminth cuticle. Cited in Advances in Parasitology IV, Edited by Ben Dawes (Academic Press, New York), 187–254.
6) Llewellyn, J. (1954): Observations on the food and the gut pigments of the polyopisthocotylea (Trematoda: Monogenea). Parasitol., 44, 428–437.
7) Nadakal, A. M. (1960a): Types and sources of pigments in certain species of larval trematodes. J. Parasit., 46, 777–786.
8) Nadakal, A. M. (1960b): Carotenoids and chlorophyllic pigments in the marine snail *Cerithidea californica* Haldeman, intermediate host for several avian trematodes. Biol. Bull., 119, 98–108.
9) Nadakal, A. M. (1963): Observations on the pigmentation of *Pallisentis* sp. (Pallisentidae: Acanthocephala), an intestinal parasite of the fish *Ophiocephalus*. Curr. Sci., 32, 220.
10) Van Cleave, H. J. and Rausch, R. L. (1950): A new species of the Acanthocephalan genus, *Arhythmorhynchus* from sand-pipers of Alaska. J. Parasit., 36, 278–283.

Acanthocephalid の1種 *Pallisentis nagpurensis* 体内色素 (carotenoid pigments) の研究

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著者らは *Pallisentis nagpurensis* 体内の黄褐色色素についてクロマトグラフィー及びスペクトロホトメーター的研究を行ない色素の本態とその由来を考察した.