

histones suggested by the work of HNLICA et al.¹³ on isolated nucleoli and BERLOWITZ and BERNSTAL¹⁴ on the anucleolate mutant *Xenopus laevis*, may be affected.

Alternatively the binding studies with ³H AMD suggest that the 'nucleolar specific' concentration of AMD might also inhibit the synthesis of quantitatively minor components of RNA on regions of the extranucleolar chromatin which are particularly sensitive to AMD binding. The transcription of such components at certain times in the cell cycle might be critical events in maintaining growth.

An independent approach to the study of nucleolar dependence can be made by the technique of microbeam irradiation¹⁵. The suggestion that the effects of AMD are mediated through injury to the nucleolus will be strongly supported if a particular action of the drug at low concentration can also be caused by specific nucleolar irradiation of similar cells¹⁶.

Résumé. Dans quelques publications récente les perturbations du cycle mitotique qui se produisent après

un traitement à l'Actinomycine D (AMD) on été attribuées à une perte de ribosomes. On se demande si on aurait considéré la fonction du nucléole et les effets de l'AMD comme trop restreintes.

A. B. RICKINSON¹⁷ and P. P. DENDY

*Department of Radiotherapeutics,
University of Cambridge,
Cambridge (England), 14 July 1969.*

¹³ L. S. HNLICA, M. C. LIAU and R. B. HURLBERT, *Science* 152, 521 (1966).

¹⁴ L. BERLOWITZ and M. L. BIRNSTIEL, *Science* 156, 78 (1967).

¹⁵ R. E. ZIRKLE, *Adv. Biol. med. Phys.* 5, 103 (1957).

¹⁶ We thank the Head of the Department, Professor J. S. MITCHELL, for his support of this work.

¹⁷ Holder of a Medical Research Council Scholarship for training in research methods.

Gonadal Pigment of Sea-Cucumber (*Holothuria leucospilota* Brandt)

The pigmentary colours¹ in the integument of echinoderms are also entirely due to the presence of naphthoquinone, carotenoid, melanin and porphyrin pigments. Most of the pigmentation occurs in the integument, but in some cases certain of the internal organs are also coloured.

Carotenoids are particularly abundant in the integument of asteroids. In echinoids, carotenoids are principally restricted to the gonads. There has been insufficient investigation of the holothuroidea² and so far as we are aware, there are no published reports on the isolation of carotenoid pigments from *Holothuroidea*.

Ovaries (500 g) containing mature eggs of *H. leucospilota* collected on the sea-shore of Kushimoto in Wakayama prefecture in July 1968 were dehydrated by treating with ethyl alcohol for several days at room temperature. The solvent was then removed by decantation and the ovaries were extracted with successive portions of acetone in the dark until the final extract was colourless. The combined reddish-orange coloured extracts were concentrated under reduced pressure under nitrogen below 40°C. The deep-red residue was dissolved in petroleum ether. A strong blue colour was obtained with antimony trichloride reagent, indicating the presence of polyene nature of the pigments. The petroleum ether solution exhibited the very characteristic intense single banded absorption spectrum (λ_{max} 472 nm) suggesting the presence of keto-carotenoid. The petroleum ether solution was washed with water to remove the acetone and the reddish petroleum ether solution was then separated into hypophasic and epiphasic carotenoids by shaking the solution with 90% methanol. The pigment was entirely extracted by the methanol. This experiment clearly confirms that the principal carotenoid pigment is unesterified xanthophyll.

From the experimental results obtained by thin-layer chromatography of original petroleum ether extract solution and partitioned methanolic solution, it was shown that the principal pigment (about 70% of total pigments) transferred to methanolic solution by the partition procedure. The methanolic solutions were concentrated under reduced pressure. The deep-red residue

was dissolved in a small amount of methanol, covered with petroleum ether, and crystallized by the addition of a little water. After 2 recrystallizations of the black-violet pigment from aqueous pyridine, 1.1 mg of glistening plates were obtained. Its properties indicate that it is unesterified astaxanthin. This was confirmed by mixed melting-point determination with an authentic specimen of astaxanthin obtained from the integument of starfish (*Asterina pectinifera* Müller and Troschel) according to the method of TSUMAKI et al.³

Astaxanthin was further characterized by its reaction with potassium hydroxide under nitrogen to form a blueish-purple enolic salt which in the presence of air almost instantaneously autoxidized into red coloured astacene, which was thin-layer chromatographically and spectroscopically identical with astacene produced in a similar way from the authentic starfish astaxanthin. From the testes (300 g) of the sea-cucumber, 0.2 mg of astaxanthin was obtained by the same procedure described above in the case of ovaries.

Furthermore, no differentiation in the pattern of carotenoid pigments in gonads of both male and female has been noted by thin-layer chromatography using different solvent systems.

Zusammenfassung. Aus den Geschlechtsdrüsen von *Holothuria leucospilota* Brandt wurde Astaxanthin extrahiert.

T. MATSUNO, T. ISHIDA,
T. ITO and A. SAKUSHIMA

*Kyoto College of Pharmacy,
Kyoto (Japan), 7 July 1969.*

¹ H. G. VEVERS, in *Physiology of Echinodermata* (Ed. R. A. BOOLOOTIAN; Interscience, New York 1966), p. 267.

² D. L. FOX and T. S. HOPKINS, in *Physiology of Echinodermata* (Ed. R. A. BOOLOOTIAN; Interscience, New York 1966), p. 277.

³ T. TSUMAKI, M. YAMAGUCHI, H. KAWASAKI and T. MUKAI, *J. chem. Soc. Japan* 75, 605 (1954).