SUMMARY

1. Lysine deficiency in rats of an initial weight of 110–140 g. produces a mild anaemia with a slightly greater reduction in haemoglobin than in red cells. It is suggested that protein deficiency may be a contributory factor in nutritional anaemia.

2. There is also a loss of protein from the liver and a hypoproteinaemia, whilst the body protein content seems to be unchanged. The importance of the liver for protein metabolism and its possible role

in connexion with the synthesis of serum proteins is discussed.

3. None of these symptoms, apart from a loss of body weight, is observed if an adequate diet is restricted in amount to produce weight changes similar to those observed in lysine-deficient animals. It is suggested that the clinical picture is produced by a general interference with protein synthesis and does not represent a specific lesion.

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The Pigments of Sponges

1. THE LIPID PIGMENTS OF THE SPONGE *HYMENIACIDON SANGUINEUM* (GRANT)

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The classification of carotenoid pigments into those present in plants, exhibiting the normal triplet spectrum, and those of animal origin, such as astacene, glycymerin and echinenone, with a single-banded spectrum, would not appear to be correct. Recently Heilbron, Jackson & Jones (1935) and Lederer (1938) have isolated animal xanthophylls possessing three absorption bands, e.g. pectenoxanthin, $C_{40}H_{54}O_3$, pentaxanthin, $C_{40}H_{56}O_5$, and sulcatoxanthin, $C_{40}H_{52}O_8$. It would seem, however, that no corresponding animal hydrocarbons have

so far been identified. Lederer (1938) has made a study of the carotenoid pigments of some fourteen species of invertebrates and has recorded his failure, save in two cases, to isolate any plant xanthophylls, a striking fact when it is remembered that the food of marine animals may include seaweed containing the xanthophylls fucoxanthin, taraxanthin and lutein. In view of this interesting observation of Lederer and in order to obtain further information with regard to the pigments of invertebrates a study has been made of the colouring matters of certain

Porifera and the present communication deals with the pigments of the sponge *Hymeniacidon Sanguineum* (Grant).

The carotenoid nature of the pigments of sponges was established by Krukenberg (1882), MacMunn (1888) and Lönnberg (1931). The former refers to their carotenoid-like and rhodophane-like pigments, the latter (probably astacene) possessing a single absorption band. These investigators observed the presence of other pigments with two or three bands resembling the carotenes or xanthophylls. Bergmann & Johnson (1933) examined the sponge *Microciona prolifera* and concluded that carotene was present but did not isolate it. More recently Karrer & Solmssen (1935) isolated the crustacean pigment, astacene, from the sponge *Axinella cristagalli* and suggested that it was also present in *Suberites domuncula*. This latter statement is at variance with that of Lederer (1938), who found that the pigments of this sponge were entirely epiphasic, even after saponification, and therefore did not include astacene. Lederer (1938) has isolated β -carotene from the sponge *Ficulina ficus*.

The sponge *Hymeniacidon Sanguineum*, used in this investigation, was obtained from the Marine Biological Station, Lough Ine, Co. Cork. The presence of large amounts of phosphatides and sterols complicated and rendered difficult the isolation of the colouring matter, a fact which might explain the comparative failure of workers in this field. After dehydration of the sponge with acetone the pigments were extracted with ether and the petroleum-soluble unsaponifiable matter partitioned with 90% methanol. The colouring matter was entirely epiphasic. Neither at this nor any subsequent stage was the presence of astacene observed. On chromatographic adsorption on alumina a number of ill-defined coloured zones was formed, the uppermost and main zone being dark red. By development of the chromatogram with mixtures of benzene light petroleum of gradually increasing eluting power it was found possible to wash through the lower zones of the chromatogram. With the use of the spectroscope as a control a fairly sharp separation into two fractions, spectroscopically distinct, was effected. From the first of these fractions α -carotene was isolated in crystalline form. The second fraction, on further purification, yielded β -carotene. From the upper deep red zone of the original chromatogram, on development with benzene, a well-defined violet zone separated and a lower orange-red zone was completely washed through. From the latter was isolated a pigment agreeing in melting-point and absorption maxima with γ -carotene. The pigment from the violet zone, when separated, and rechromatographed twice on aluminium oxide, showed an intense absorption band at $488m\mu$ in carbon disulphide and melted at

192–193°. It would appear to be identical with echinenone, the first animal-provitamin A isolated by Lederer (1935) from the sea-urchin *Strongylocentrotus lividus*. The residual upper zone of the original chromatogram consisted of oxidized pigments or their decomposition products, showing only indefinite absorption spectra. It was not found possible to isolate any definite substance therefrom.

The demonstration of the presence of echinenone in a sponge provides a chemical link between the otherwise unrelated Porifera and Echinodermata. It would appear that this is the first occasion on which γ -carotene has been isolated from invertebrates.

An interesting speculation can be made from the occurrence of astacene in Karrer's deep-water sponge in contrast to the isolation of less oxygenated, non-acidic pigments in the present instance from a shallow-water sponge. The difference may be connected with the penetration of light which may give the pigments a different phytochemical significance in each case. Of interest in this connexion is the influence of light on the pigmentation of the prawn, *Palaemonetes vulgaris* (Brown, 1934). In water, with a white background, the red pigment (astacene) of this animal and its blue chromoprotein are rapidly destroyed in the light. This carotenoid is formed, however, when the background is black.

EXPERIMENTAL

The red sponge *Hymeniacidon Sanguineum* (1 kg. dry weight) was collected in March. After freeing from mussels and algae some of the water was expressed by hand. The sponge was immediately chopped into small pieces, covered with pure acetone, and the whole allowed to remain overnight. The solvent, on being filtered off, was only faintly coloured and was neglected. On repeating once more the above operation, the acetone filtrate assumed a light yellow colour. The dry sponge was now covered with ether (peroxide-free) and the whole left overnight in a dark place. After filtering, the extraction with ether was repeated three times, the final ether extract being only faintly coloured. The united deep-red ether extracts (6 l.) were dried with anhydrous Na_2SO_4 and evaporated under reduced pressure, in an atmosphere of CO_2 , the temperature being kept below 30°. The reddish butter-like residue was next saponified by heating at 40° for 3 hr. with 10% methanolic potash, in an atmosphere of nitrogen.

To test for the presence of astacene a small portion of this solution was withdrawn and covered with a layer of light petroleum. On acidification by addition of dilute acetic acid no separation of solid at the interface was observed. The main portion was diluted with water and extracted three times with light petroleum (b.p. 60–80°). The alkali layer was coloured olive-green due, probably, to the presence of chlorophyll and its decomposition products. The deep-red light-petroleum layer was washed with water until free from alkali and methanol and dried with anhydrous Na_2SO_4 . As it was found necessary to remove accompanying sterols at this stage, the light-petroleum extract

was concentrated somewhat and placed in the ice-chest overnight. The bulky crystalline mass which separated was filtered off and the filtrate partitioned with 90% methanol. In this way a red epiphasic and a faint yellow hypophasic layer were obtained.

Light-petroleum phase. The light-petroleum layer (1 l.) was freed from methanol by repeated washing with water, dried with anhydrous Na_2SO_4 and adsorbed, in two portions, on two columns (20×5 cm.) of aluminium oxide (Merck). The chromatogram consisted of a series of ill-defined zones: (1) deep-red (2 cm.), (2) orange-red, (3) orange-yellow. On development with a 2% solution of benzene in light petroleum an orange-yellow filtrate was obtained and this was collected until a distinct change in its spectral readings was observed (filtrate 1). At this stage further development was continued with light petroleum containing 10% of benzene, when a second orange-coloured filtrate was collected. Here again development with this mixed solvent was continued until a change in absorption spectrum took place (filtrate 2). The upper zone of the chromatogram, which was still deep red, had now increased in depth to 3 cm. On further development with benzene a violet zone gradually made its appearance and a lower reddish band moved into the filtrate (filtrate 3). Washing with benzene was continued until the violet zone had become well defined. The violet zone when separated, eluted, and rechromatographed twice on aluminium oxide furnished a small uniform violet and a lower orange-red zone. Elution of the violet zone and repeated crystallization of the eluate from benzene-methanol furnished a pigment consisting of bluish violet needles melting at $192-193^\circ$ and showing one broad band at $488 \text{ m}\mu$ in carbon disulphide. It would appear to be identical with echinenone, first isolated by Lederer (1935), although the latter states that echinenone exhibits, in addition to the broad band at $488 \text{ m}\mu$, two very faint bands at 520 and $450 \text{ m}\mu$ in carbon disulphide.

FILTRATE 1. This was evaporated to dryness *in vacuo*, the residue dissolved in light petroleum ($60-80^\circ$) and the solution shaken with a small quantity of fuller's earth (Kuhn & Brockmann, 1931). The filtrate, on concentration to small bulk, deposited a pigment which on recrystallization from the same solvent was obtained as violet-coloured prisms

melting at $182-184^\circ$, with absorption maxima in carbon disulphide at $511, 478, 448 \text{ m}\mu$ and in light petroleum at $478, 448 \text{ m}\mu$, in close agreement with the recorded data for α -carotene.

FILTRATE 2. This was brought to dryness, the residue dissolved in light petroleum and chromatographed on air-dried calcium hydroxide. An upper red zone and a lower yellow zone (α -carotene) were formed. Elution of the red zone furnished a substance which on repeated crystallization from benzene-methanol gave glistening, reddish brown prisms of β -carotene, melting at $181-182^\circ$, with absorption maxima in carbon disulphide at $520, 484, 462 \text{ m}\mu$, and in light petroleum at $484, 452, 426 \text{ m}\mu$.

FILTRATE 3. This, on evaporation, left a red, wax-like residue which was dissolved in light petroleum and rechromatographed on aluminium oxide. The upper red zone was eluted with light-petroleum methanol and the eluate concentrated to small bulk. On standing, the pigment separated in reddish needles which were still admixed with a colourless impurity. To remove the latter the pigment was extracted twice with boiling methanol and crystallized three times from benzene-methanol (2:1), when γ -carotene was obtained in dark red prisms, melting at $176-177^\circ$, with absorption maxima in carbon disulphide at $534, 496, 463 \text{ m}\mu$ and in chloroform at $508, 474, 446 \text{ m}\mu$.

Methanol phase. This was diluted with water and extracted with light petroleum, all the pigment passing into the petroleum layer. This was washed free of methanol, dried with Na_2SO_4 , and chromatographed on aluminium oxide. A faint reddish zone was formed which, on elution, gave a minute quantity of pigment with the absorption characteristics of γ -carotene.

SUMMARY

1. The pigments of the sponge *Hymeniacidon Sanguineum* (Grant) have been examined and found to be entirely epiphasic.

2. The carotenoids isolated and identified were α -, β - and γ -carotenes and echinenone.

3. No evidence of the presence of the crustacean pigment, astacene, was obtained.

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