Studies on the Sea Anemone *Gyrostoma* sp.

LIPIDS OF *GYROSTOMA* SP.

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Very little is known about the lipid content of the sea anemone, particularly of those found in tropical waters. This work was undertaken with a view to determining the concentration of lipids and allied substances in the sea anemone, *Gyrostoma* sp., found along the shores of Bombay.

EXPERIMENTAL

The sea anemones were collected during low tide on the beach near the Institute and were preserved in a live condition in aquaria till used for extraction.

Extraction and analysis

The sea anemones were dissected and as much water as possible was removed by drying them between filter papers. Some anemones were used whole for extraction, and in the others the gonads, mesenteries and tentacles were removed and extracted separately. In all these determinations, a small part of the anemone was always dried in an oven at 110° to determine the moisture content.

Either the whole anemone (20–25 g.) or the different organs (5–10 g.) were extracted with ethanol and ether by the method described by Johnson, McNabb & Rossiter (1948), with the difference that the quantities of ethanol and ether for each extraction were adjusted to make the final volume up to 4 l. The same procedure of Johnson *et al.* (1948) was followed for removing the solvent and precipitating the phosphatides in three separate 400 ml. samples of the ether-ethanol extract. The phospholipids were made up to 25 ml. with methanol-ether (1:1, by vol.) and the acetone washings up to 50 ml.

All analyses were done in triplicate.

Free and total cholesterol. These were estimated in 2 ml. of the acetone solution by the modified method of Schoenheimer & Sperry (1934), incorporating the improvements suggested by Sperry & Brand (1943) and Sobel & Mayer (1945). The colour was read in a Beckman spectrophotometer (DU Model) at 620 m μ .

Cerebrosides. The method of Brand & Sperry (1941) was used, the galactose liberated on hydrolysis being estimated by the procedure of Miller & Van Slyke (1936). Six 50 ml. samples were taken from the original ether-ethanol solution and, of these, three were hydrolysed and the others treated in exactly the similar way except that hydrolysis was omitted. The quantity of cerebrosides was calculated from the following equation:

$$\text{Wt. of cerebrosides (mg.)} = 4.55 \times 0.147(A - B) - (C - D)$$

in which *A*, *B*, *C* and *D* are the volumes of 3 mm ceric sulphate solution required to titrate the hydrolysed lipid

sample, hydrolysed blank, unhydrolysed lipid sample and unhydrolysed blank respectively. The factor 4.55 was used on the assumption that approximately equal quantities of phrenosin and kersin were present. The factor for phrenosin is 4.6 and for kersin it is 4.5.

Phospholipids. These were estimated in the methanol-ether solution by the method of Hack (1947), in which it has been observed that the mono-aminophospholipids lecithin and cephalin are completely hydrolysed by N-KOH in 18 hr. at 37° and that no hydrolysis of sphingomyelin takes place under these conditions. The total acid-soluble P is a measure of the lecithin and cephalin present (Schmidt, Benotti, Hershman & Thannhauser, 1946) and choline a measure of the lecithin (Hack, 1947). The difference between the total P and the total acid-soluble P in the hydrolysate is a measure of sphingomyelin, and the difference between the molecular equivalents of mono-aminophospholipid and choline gives the molecular equivalent of cephalin.

For the estimation of total P, 1 ml. was taken from the 25 ml. volumetric flask containing the methanol-ether solution of the phospholipids and the total P determined by the method of Sterges, Hardin & MacIntire (1950). The phospholipid was calculated on the assumption that it contained 4.0% of phosphorus.

The remaining 24 ml. of the methanol-ether solution was evaporated under reduced pressure at 60° to a volume of 0.2 ml. After the addition of 5 ml. of N-KOH the tube was stoppered and shaken at 37° for 18 hr. To 0.5 ml. of this solution, pipetted into a centrifuge tube, 0.1 ml. of 5N-HCl and 3 ml. of 10% (w/v) trichloroacetic acid were added. The centrifuge tubes were stoppered, shaken and allowed to stand at room temperature for 1–2 hr. After centrifuging, the solution was filtered through a Whatman no. 44 filter paper and the total P (mono-aminophospholipid P) estimated in 2 ml. of the trichloroacetic acid filtrate (representing 1.28 ml. of the methanol-ether solution) by the method of Sterges *et al.* (1950). The mono-aminophospholipid was calculated on the assumption that it contained 4.0% of phosphorus.

Lecithin. An estimate of the lecithin present was obtained by determining choline in the remainder of the hydrolysate according to the method of Glick (1944). The colour density was measured in the Beckman spectrophotometer at 526 m μ . Lecithin was calculated as choline (as choline chloride) $\times 5.56$ in the filtrate.

Kephalin. This was taken as representing all the non-choline-containing phospholipids and was calculated as the difference between the mono-aminophospholipid and lecithin.

Sphingomyelin. This is a diaminophospholipid and was calculated as the difference between the total phospholipid and the mono-aminophospholipid.

Table 1. *Lipid composition of the whole anemone, gonads, mesenteries and tentacles of the sea anemone, Gyrostoma sp.*

Each value is the mean of three determinations made on each of four batches of material; standard deviations are also given.

	Cholesterol					
	Cerebroside		Free		Ester	
	% of dry wt.	% of total	% of dry wt.	% of total	% of dry wt.	% of total
Whole anemone	0.83 ± 0.03	14.78 ± 0.77	0.23 ± 0.02	4.07 ± 0.40	0.25 ± 0.03	4.37 ± 0.45
Gonads	0.59 ± 0.03	6.79 ± 0.45	0.80 ± 0.06	9.18 ± 0.48	0.43 ± 0.04	4.89 ± 0.33
Mesenteries	0.89 ± 0.03	16.04 ± 0.30	0.23 ± 0.05	4.10 ± 0.78	0.29 ± 0.08	5.25 ± 1.29
Tentacles	0.12 ± 0.02	6.38 ± 0.38	0.09 ± 0.01	4.60 ± 0.44	0.05 ± 0.01	2.72 ± 0.49
	Phospholipid					
	Lecithin		Sphingomyelin		Kephalin	
	% of dry wt.	% of total	% of dry wt.	% of total	% of dry wt.	% of total
Whole anemone	3.08 ± 0.11	55.08 ± 1.33	0.25 ± 0.01	4.42 ± 0.19	0.97 ± 0.10	17.30 ± 1.65
Gonads	4.86 ± 0.13	55.62 ± 1.54	0.18 ± 0.02	2.12 ± 0.25	1.87 ± 0.13	21.40 ± 1.24
Mesenteries	2.83 ± 0.09	50.68 ± 1.44	0.39 ± 0.04	7.10 ± 0.90	0.94 ± 0.07	16.86 ± 0.85
Tentacles	1.12 ± 0.14	60.20 ± 3.87	0.12 ± 0.02	6.46 ± 0.40	0.37 ± 0.11	19.64 ± 4.16

RESULTS

The mean concentration of cerebroside, cholesterol, total phospholipids, the individual phospholipids lecithin, kephalin and spingomyelin and the lipid fractions mentioned above expressed as a percentage of the essential lipid, i.e. as a percentage of the sum of the total cholesterol, cerebroside and total phospholipids, as well as the standard deviation, are presented in Table 1. The results expressed are the means of the readings obtained with four different batches of material. Each of the four analyses was carried out in triplicate.

From Table 1 it can be seen that the gonads contain most phospholipid, the tentacles containing the least. The mesenteries contain only slightly less phospholipid than the gonads. Cerebroside are found most in the mesenteries and least in the tentacles. Cholesterol, both combined as well as free, is contained more in the gonads than in any other organ.

The concentration of lecithin and kephalin is greater in the gonads than in any other organ. However, the sphingomyelin content of the gonads is very low, the mesenteries having the greatest concentration. The tentacles contain the least amount of phospholipids.

When expressed as a percentage of the essential lipid, i.e. as a percentage of the sum of the total cholesterol, cerebroside and total phospholipid, it is seen that the tentacles contain more phospholipid as lipid than any other organ. The gonads contain relatively more cholesterol and cerebroside. Of the individual phospholipids, lecithin forms the maximum percentage in the tentacles. The sphingomyelin content on this basis is greatest in the mesenteries and least in the gonads.

DISCUSSION

There are almost no reports in the literature on the distribution of phospholipids in the different organs of the sea anemone.

Cholesterol has been shown to be a precursor of progesterone, one of the female sex hormones (Bloch, 1945). The presence of such high concentrations of cholesterol in the gonads, the reproductive organs of the sea anemone, is therefore significant.

The concentration of certain lipid constituents, e.g. sphingomyelin and cerebroside, may indicate how much of the lipid present in the tissue is of neural origin (Kaucher, Galbraith, Button & Williams, 1943). There is a possibility that the mesenteries, which contain the maximum concentration of these two constituents, have much of their lipid of neural origin.

An important function of the phospholipids is to carry choline as well as fatty acids. In the higher animals choline plays an important part in the mobilization of fat from the liver. The gonads seem to be the main depots of fat in the sea anemone. Choline may be useful in the carrying of fat from the gonads to the other organs. The presence of such high concentration of choline (lecithin) in the mesenteries is significant in that it may be helpful in the digestion of the fats.

SUMMARY

1. The concentrations of cerebroside, free and total cholesterol, total phospholipid, lecithin and sphingomyelin have been determined in the whole anemone, *Gyrostoma* sp., as well as in its mesenteries, gonads and tentacles.

2. A great variation in lipid content of the various organs of the sea anemone was found, according to the physiological functions they perform, the gonads containing the maximum amount of essential lipid.

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Studies of Sebum

7. THE COMPOSITION OF THE SEBUM OF SOME COMMON RODENTS*

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In order to study the mechanism by which the various components of sebum are formed a suitable laboratory animal was required. Ideally such an animal should have a sebum whose composition closely resembles that of man, but, with the exception of the sheep, little is known about the composition of the hair lipids or sebum of animals. A study was therefore made of the composition of the sebum of the common laboratory animals, namely the guinea pig, mouse, rabbit and rat. The purpose of the investigation was to find how widely the composition of the sebum differed from that of man, so that their uses and limitations as experimental animals could be assessed. A preliminary report of part of this work has already been published (Wheatley, 1953).

EXPERIMENTAL

Materials

In order to obtain a material which would compare with the sebum collected from human subjects the following method of collection was adopted. The mouth and anal regions of the freshly killed animals were cleaned with chloroform, and the body was suspended horizontally over a glass tank. A quantity of acetone (previously twice distilled in all-glass apparatus) was poured over the back of the animal in such a manner that as little as possible flowed over the ears, mouth

and anal regions. The acetone which collected in the glass tank was poured back over the animal several times and the animal finally washed with a fresh quantity of acetone. With mice a slightly different procedure was adopted, owing to their smaller size and the small amount of sebum collected from each animal. These animals were simply held by the tail, after the mouth and anal regions had been cleaned with chloroform, and were immersed in a beaker containing acetone. The acetone extract from a number of animals was evaporated to dryness, and the residue dissolved in chloroform. This solution was filtered to remove cellular and other debris and then evaporated to dryness. The amount of material obtained by this procedure from a single animal was approximately as follows: guinea pig 250 mg., mouse 10 mg., rabbit 250 mg., rat 100 mg.

Analytical methods

Chemical constants. Iodine values, acid values, phosphorus and nitrogen were determined by the methods referred to in earlier work (Wheatley, 1954). Cholesterol and 'fast-acting' sterols were determined by the method described by Moore & Baumann (1952). The acrolein method (MacKenna, Wheatley & Wormald, 1950) was used to test for the presence of glycerol.

Fractionation. The fractionation into free fatty acids, combined fatty acids and unsaponifiable matter followed the procedure already described for human sebum (MacKenna *et al.* 1950).

Gas-liquid chromatography. The mixed fatty acids were esterified with methanol and sulphuric acid, and the mixed esters applied directly to the gas chromatogram with a micro-pipette. The general procedure adopted has been

* Part 6: James & Wheatley (1956).