PEARL-LIKE CONCRETIONS (CALCULI) FOUND IN THE STOMACH OF CARTILAGINOUS AND BONY FISHES FROM THE ANDAMAN SEA.[1]

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(Communicated by Dr. S. L. Hora, D.Sc., F.N.I.)

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(Plate II.)

The term *pearl* is usually applied to certain secretion products of oysters, mussels, clams and such other bivalve molluscs with a nacreous layer to the inside of the shell, and even of univalve molluscs such as *Strombus*, *Xancus* (=*Turbinella*), etc. in which the innermost layer of the shell is porcellaneus. The pearls proper are rounded masses of shell-substance made up of concentric layers laid down around a nucleus. Some secretion products of animals other than molluscs, such as fish, crustaceans, and mammals, very similar to true pearls in appearance, but differing in chemical composition, have also been known for a long time. They are termed *concretions* or *calculi*.

The occurrence of pearl-like concretions in animals other than molluscs is recorded in literature in various scattered works, and in the following few paragraphs a very brief summary of the records is given. In regard to concretions in fish the author was able to find very few references in literature from the 18th century up to the present day. Dr. E. W. Gudger of the American Museum of Natural History, New York, has kindly looked through his exhaustive card catalogue on the subject and confirmed that no references to concretions in fish occur in recent literature other than those recorded in Bashford Dean's '*Bibliography of Fishes*' (1916–23). It was, therefore, thought that the present record of the occurrence of concretions in fishes from the Andaman waters would be of some interest, and would lead to further observations on fish, both marine and freshwater, in which calculi may be present.

In connection with the investigations on the bionomics of *Trochus niloticus* Linn. in the Andamans, the author had the opportunity of examining the stomach contents of various predacious fishes. On two separate occasions in the course of his cruise on the Japanese fishing boats licensed to fish shells of *Trochus* in the Andamans, two species of large fish, the cartilaginous *Zygaena blochii* Cuvier (Hammer-headed Shark) and the bony *Caranx* (*Caranx*) *melampygus* Cuv. & Val., locally known as 'Cocari' were caught on line in the Cleugh

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Passage, N. Andamans, and in the vicinity of Neill I. (Ritchie's Archipelago) respectively. The stomachs of both the fishes contained the remains of bony fishes, and pearl-like concretions of various sizes lying loose, but mixed up with the partially digested contents of the stomach. The concretions did not appear to have any organic connection with the walls of the stomach.

Before dealing with the description of the concretions, it would be useful to refer to the few records in literature of the occurrence of calculi in fishes and other animals. Collinson (1748) recorded the occurrence of ' stones ' in the Sturgeon of the Volga region, known locally at Astrakhan as ' Belluga '. They were oval, globular, or roundish, with unequal depressions, of a yellowish white colour, and a polished surface. The ' stones ' consisted of concentric coats firmly adhering to one another, formed round a nucleus which, judging from its colour, hardness, and texture, appeared to be a heterogenous substance. A number of shining rays seemed to diverge regularly from the central nucleus to the circumference resembling the striated spicula of antimony. It was not known from which part of the Sturgeon's body the ' stones ' were taken. Collinson thought it a morbid product like the stone in the bladder of human beings. Its chemical composition was not determined. The ' Belluga-stone ' was thought to be very scarce as not even one in a thousand fish was found to contain it.

Oseretskovsky (1786) reported that in 1783 calculi were found in a species of Sturgeon from Astrakhan. The calculi were elongate-oblong, smooth, and heart-shaped bodies with a few depressions and grooves on the surface.

Fourcroy and Vauquelin (1807) recorded the remarkable occurrence of a fish-hook in the stomach of a fish coated by concentric layers of a dull, yellowish material, smooth and brilliant, and composed of calcium carbonate, calcium phosphate, and some gelatinous organic matter. The material and manner of deposition on the fish-hook seemed to show that the former was of the nature of a concretion secreted by the tissues of the fish around the nucleus provided by the irritant fish-hook.

Delachanel and Mermet (1874) recorded a large intestinal calculus weighing 150 grammes from a Sturgeon caught at Astrakhan. The substance of the calculus was arranged in concentric layers round a nucleus, and was composed of 84 parts of ' phosphate bibasique ', 2 parts of water and 15 of organic matter with some traces of acid sulphuric, lime, magnesium, aluminium and oxide of iron. A trace of phosphate of lithin was also found.

Tower (1902) reported the occurrence of several calculi in the gall-bladder, gall-duct, and in the tissues between the liver and the intestines in the fish, *Cynoscion regalis*, at Woods Hole, U.S.A. They were rounded, oval, or oblong bodies, 5–15 mm. in diameter, nodulated or smooth on the surface, and consisting of concentric layers round one or more nuclei. The composition of the calculi was found to be as follows : cholesterin and fat 2·85%, mineral 3·65%, bilirubin 16·14%, nucleoalbumin 65·59%, water 11·52%, material soluble in water, a trace. The total ash of calculi was 4·32%. The fish from which the

calculi were obtained were apparently normal when taken from water. The high percentage of nucleo-albumin in these calculi showed that they were different from the gall-stones of other animals.

Herrick (1910) recorded the occurrence of a ' pearl ' [1] from the claw of a lobster from Long I., U.S.A. It was nearly spherical, 11 mm. in diameter, and of a light buff colour. In structure, texture, colour, and specific gravity it agreed well with the shell of the lobster, and was originally connected with the shell of the claw. The author expressed the opinion that the ' pearl ' in question was not of the type produced by a pearl-secreting mollusc in which the ' deposits of shell-substance are successively added by epithelial cells having a different relation to the shell, which is never cast off ' as in the lobster in the process of moulting.

Chopra (1930) detailed the occurrence of a ' pearl ' in a Peneid prawn from Calcutta. The exact position of the ' pearl ' in the body of the prawn was not observed. It was presumed that the ' pearl ' had been formed in the thick abdominal muscles from the fact that the existence of the ' pearl ' was known when the prawn was eaten ; and the only part of a prawn valued as food is its abdomen. The ' pearl ' was spherical in shape with slight protuberances and hollows, slightly less than 3 mm. in diameter, practically colourless with a somewhat pearly lustre, transparent and without a nucleus. It was formed of close concentric layers with extremely fine meridional striations. Its specific gravity was 1·32, refractive index 1·558, and hardness 2·5. The ' pearl ' seemed to be somewhat porous. The author had " no doubt that the ' pearl ' was made of chitin, similar to the hard shell of the prawn ", as the specific gravity and refractive index agreed closely with those of chitin precipitated from its solution in strong acids.

The ' pearls ' from the Andaman fishes may now be described. Those from the Hammer-headed Shark, *Zygæna blochii*, were three in number, two large (9·0 and 9·2 mm. in diameter) and one small (3·2–3·5 mm. in diameter) weighing 0·58 gramme and 0·017 gramme respectively. When examined fresh they showed several flakes of a transparent bluish-green material with fine parallel striations (0·01 mm. between striæ), and easily peeled off from the inner colourless part of the concretion. It is this inner part, hard, smooth, wax-like in appearance, which is comparable to a precious pearl, but lacks the polish and sheen of the latter. It consists of a number of close concentric layers, each about 0·025 mm. thick (pl. II, fig. 3). A very minute thin space may also be discerned between some of the layers. The surface of each of the layers is minutely striated. One of the large ' pearls ' has a nucleus, while the other two have none. Judging from the colour, and the striations of the nucleus which can be observed under the microscope, there seems little doubt that it is a minute flake of the pale bluish-green material which forms the outer

[1] Where the word ' pearl ' is used, pearl-like concretion or calculus is meant.

layers of the 'pearl'.[1] The surface of the 'pearl' bears meridional striations, and in addition fine cracks, also meridional. In the larger 'pearl' these cracks are more numerous on the surface giving it the appearance of a mosaic. A slightly excentric cross-section of the 'pearl' near the nucleus (pl. II, figs. 3 and 4) shows the disposition of the layers and a hollow (pl. II, fig. 4) which lodges the central portion of the 'pearl' bearing the nucleus.

The physical properties of the outer bluish-green flakes and the inner 'pearl' as determined by Dr. J. A. Dunn, Curator of the Geological Survey of India, are tabulated below :—

	Flakes.	'Pearl.'
Specific gravity (when dry)	1·387	1·295
Specific gravity after 3 days in water ..	1·396	1·316
Hardness	2·5	2·5
Refractive index	1·551–1·553 (varying with fragments).	1·553–1·556 (varying with different directions).
Colour	Pale-bluish green, transparent.	Colourless.
	Perfectly isotropic.	Anisotropic (Extinction at an angle to the striations, about 30°).
		Dark cross between crossed nicolls.

The chemical composition of the 'pearls' and the flakes has not been fully determined, but the results of the quantitative and qualitative analysis both by micro- and semi-micro-tests obtained by Dr. D. Chakravarti of the Chemistry Department, University College of Science, Calcutta, are given below :—

	'Pearl.'		Flakes.	
	Test Semi-micro-	Test micro-	Test Semi-micro-	Test micro-
Weight of substance ..	0·02012 gm.	0·002828 gm.	0·01005 gm.	0·002472 gm.
CO_2	0·03536 ,,	....	0·01728 ,,	....
H_2O	0·01139 ,,	....	0·00636 ,,	....
C-percentage ..	47·93	....	46·89	....
H- ,, ..	6·292	....	7·034	....
N_2 at 763 mm. & 23°·5C.	....	0·36162 C.C.	....	0·30968 C.C.
N-percentage ..	....	14·77	....	14·4

Loss on ignition ..	99·12%
Substance	0·2505 gm.
Wt. of ash	0·0022 ,,

[1] Hornell (1922) says that precious pearls may be induced by the irritation of foreign bodies, or by nuclei of periostracal substance derived from the oyster's own tissues. Large cyst pearls are formed by the former, and small ones by the latter. The 'fish-pearls' are presumably formed in the same way, i.e. by the irritation set up by any foreign particle or by the loose outer flakes of other 'pearls'.

The qualitative test is shown below in a graphic form [1]:—

'Pearl' (powdered and extracted with chloroform)

Solution (evaporated). Slight *traces of* a substance which responds to Liebermann's test for *cholesterol*. *Fat*, if present at all, is present only *in traces*.	Residue (shaken with water and left for 2 days).
Solution (evaporated). Only traces of a substance which responds to Xanthoproteic test. The solution does not reduce Fehling's solution, hence *gluco-proteins* are *absent*.	Residue (shaken with cold absolute alcohol).
Solution (evaporated). Practically no residue.	Residue (Hydrolized with hydrochloric acid, when Fehling's solution is reduced). This shows the *presence of chitin*, which is probably the main constituent of the 'pearl'.

The 'pearls' taken from the stomach of ('Cocari') *Caranx* (*Caranx*) *melampygus* Cuv. & Val. (pl. II, figs. 5–13) were four in number, one large, 6·5–6·8 mm. in diameter, and three small, 4·4, 4·2, 3·4 mm. in diameter, weighing respectively 0·23 gm., 0·05 gm., 0·047 gm., and 0·025 gm. When removed from the stomach [2] they all had a coating of sticky white tissue (pl. II, figs. 5–8) easily removed by rubbing between the forefinger and thumb. Flakes of whitish material constitute the outer layers of the 'pearl', but are not so well defined as in the previous lot. The 'pearls' are very similar in appearance to those taken from *Zygæna blochii* in transparency, colour and texture. The striations are very regularly meridional, and there are fewer cracks on the surface. An equatorial section through one of the 'pearls' shows a flake of dark-grey striated material as the nucleus in the centre. Dr. J. A. Dunn has been kind enough to determine the following physical properties: Specific gravity 1·290, after three days in water 1·340; hardness 2·5; refractive index 1·556–1·557. The 'pearls' have a very low birefringence, and are anisotropic. Their chemical composition, which has not been investigated, is presumably very similar to that of the 'pearls' from the shark.

The two lots of 'pearls' from the two species of fish from the Andamans seem to agree very closely in specific gravity, refractive index, and hardness with the 'pearl' described by Chopra (*loc. cit.*) from a prawn, as seen in the comparative table below showing the size, weight, and physical properties of 'pearls' known since 1930. The facts relating to the Lobster 'pearl' recorded by Herrick (*loc. cit.*) are also included in the table.

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[1] Spectrographic examination of the 'pearl' and the green flakes does not show any trace of the metals. The author has to thank Dr. K. S. Krishnan, Mahendra Lal Sircar Professor, Indian Association for the Cultivation of Science, for kindly undertaking the spectrographic examination of the material.

[2] A dark brown sac associated with the liver and the pancreas of this fish contained a serrated dorsal or pectoral spine of a Siluroid fish belonging probably to the genus *Arius*. I am indebted to Mr. D. D. Mukerji for the identification of the predatory fish as well as its prey.

	Diameter in millimeters.	Weight in grammes.	Specific gravity		Refractive index.	Hardness.	Dark cross between crossed nicolls.
			In dry condition.	After 3 days in water.			
'Pearls' from 'Cocari'-1936.	*Large* 6·5–6·8 *Small* 4·4, 4·2, and 3·4.	*Large* 0·23 *Small* 0·05, 0·047 0·025.	1·290	1·340	1·556–1·557	2·5	Present.
'Pearls' from Hammer-headed shark.-1935.	*Large* 9·0–9·2 *Small* 3·0–3·5	*Large* 0·580 *Small* 0·017	1·295	1·316	1·553–1·556	2·5	Present.
'Pearl' from Peneid prawn-1930.	Slightly less than 3.	0·0174	....	1·320	1·558	2·5	Present.
'Pearl' from Lobster-1910.	11·0	....	..	1·45	..	3·0	..

The 'pearl' from the lobster described by Herrick (1910) seems to differ from all the three both in specific gravity and in hardness, the only two physical properties determined by him. There appears to be some variation in the specific gravity, and in the refractive index of the chitin from the integument and other organs of various animals, such as Insects, Arachnids, Worms, Crustaceans and Molluscs, as shown by Sollas (1907), but the range of variation seems to be restricted, with specific gravity from 1·392 to 1·430, and refractive index from 1·544 to 1·557. Although the 'fish-pearls' have a specific gravity below the range indicated by Sollas, the outer flakes conform very nearly to that of chitin precipitated from its solution in strong acid, and as shown on p. 96, the percentage proportions of the chemical ingredients, e.g. Carbon, Hydrogen and Nitrogen, are almost the same in the 'pearls' as in the flakes. The qualitative analysis shows very slight traces of cholesterol and fat, and the unmistakable presence of chitin which seems to be the main constituent of the 'pearl'.

The next point to be considered is the relation of the 'pearl' to the animal from which it was taken, whether the 'pearl' was a product of the tissues of the bony fish that had been eaten, or of the tissues of the predatory fish. This point was difficult to determine as the contents of the stomach of the fish, when examined soon after capture, were more or less in a digested state, and the 'pearls' themselves were found lying loose along with the bones of the fish digested. The 'pearls' could not have belonged to a Crustacean or a Mollusc, as no trace of the hard shell of either animal was found in the stomach.[1] No

[1] In certain Gymnodontid fishes examined last year, the stomach contained fragments of the shell of *Turbo*, and of the carapace and appendages of a species of crab.

organic connection between the tissues of the stomach of the predatory fish and the ' pearls ' has been found as in the case of the Lobster ' Pearl ' recorded by Herrick (1910). The ' fish-pearls ' described in this paper may, however, have been formed like the calculi in the gall-bladder and in the tissues between the liver and the intestines of the fish, *Cynoscion regalis*, as recorded by Tower (1902). The chemical composition of the calculi from *Cynoscion regalis* seems to be very different from that of the ' fish-pearls ' which consist of pure chitin and a trace of cholesterol. Assuming that the ' pearls ' from the Andaman fishes had been secreted by some of the structures associated with the digestive organs of either the pedatory fish or its victim, the question is, how chitin came to be secreted by the internal organs. Halliburton (1885) pointed out that chitin has been recorded to occur in epiblastic as well as in mesoblastic structures, as for instance, in the nerves of Crustacea, in the cartilage, liver and other mesoblastic structures of the cuttle-fish, the King-crab, etc. By a qualitative analysis he showed conclusively that chitin is present in the liver of *Limulus*, presumably in the connective tissue of that organ rather than in the liver-cells. It is, therefore, probable that the ' fish-pearls ' recorded here have been secreted by the gall-bladder or by the connective tissue of the liver of the fish. Similarly, the calculi recorded by Collinson (1748), and Delachanel and Mermet (1874) from the Sturgeon of the Volga region may have been the products of secretion of the connective tissue of the liver, but except in the general form and structure they do not seem to have much in common with the ' fish-pearls ' recorded here, which are of a purely chitinous nature. The ' prawn-pearl ' recorded by Chopra (1930) appears, from its physical properties, to be of the same type as the ' fish-pearls ', but its chemical composition was not studied.

In the preparation of this paper the author has received considerable encouragement from his friend, Dr. S. L. Hora, to whom his best thanks are rendered. Dr. J. A. Dunn of the Geological Survey of India, and Dr. D. Chakravarti of the Chemistry Department, University College of Science, Calcutta, have determined the physical and chemical properties of the ' fish-pearls ', while Dr. K. S. Krishnan has examined the ' pearls ' spectrographically. To these gentlemen, the author wishes to record his grateful thanks.

Summary.

1. The scarcity of records on the occurrence of pearl-like concretions or calculi from animals other than molluscs, such as fish, Crustaceans, etc., is pointed out, and a brief résume of the literature on the subject is given.

2. Pearl-like concretions from the stomach of a hammer-headed shark and of a Carangid fish (' Cocari ') from the Andamans are recorded for the first time from the Indian seas.

3. An account of the structure of the Andaman fish ' pearls ' and of their physical and chemical properties is included. The substance of the fish ' pearls ' is identified with chitin.

4. The size, weight, and physical properties of the Andaman fish 'pearls', and of the Crustacean 'pearls' known since 1910 are shown in a tabular form, and the very close resemblance in physical properties of the former to the Calcutta prawn 'pearl' is pointed out.

5. The formation of chitin in the external and internal structures of various animals is discussed, and the suggestion is made that the chitinous 'pearls' from the Andamans may have been formed in the gall-bladder or in the connective tissue of the liver of the predatory fish or its prey.

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Explanation of Plate II.

'Fish-pearls' from the Andamans.

Figs. 1 and 2. Large and small 'pearls' from the stomach of *Zygœna blochii* after removal of the outer bluish-green flakes.

Figs. 3 and 4. The large 'pearl' has been cut slightly excentrically. Fig. 3 shows the larger hemisphere and Fig. 4 the smaller. The latter has a hollow in the centre which contained the nuclear part of the 'pearl'. The concentric layers of chitin are clearly seen in the sections.

Figs. 5 and 6–8. Large and small 'pearls' from the stomach of *Caranx* (*Caranx*) *melampygus* covered by layers of sticky white tissue.

Figs. 9–11. Small 'pearls' (Figs. 6, 7, 8) from *C. melampygus* with the sticky white tissue removed.

Figs. 12-13. Large 'pearl' from *C. melampygus* showing the cut surfaces after removal of the sticky white tissue. The section is equatorial.

All the figures are magnified three times.

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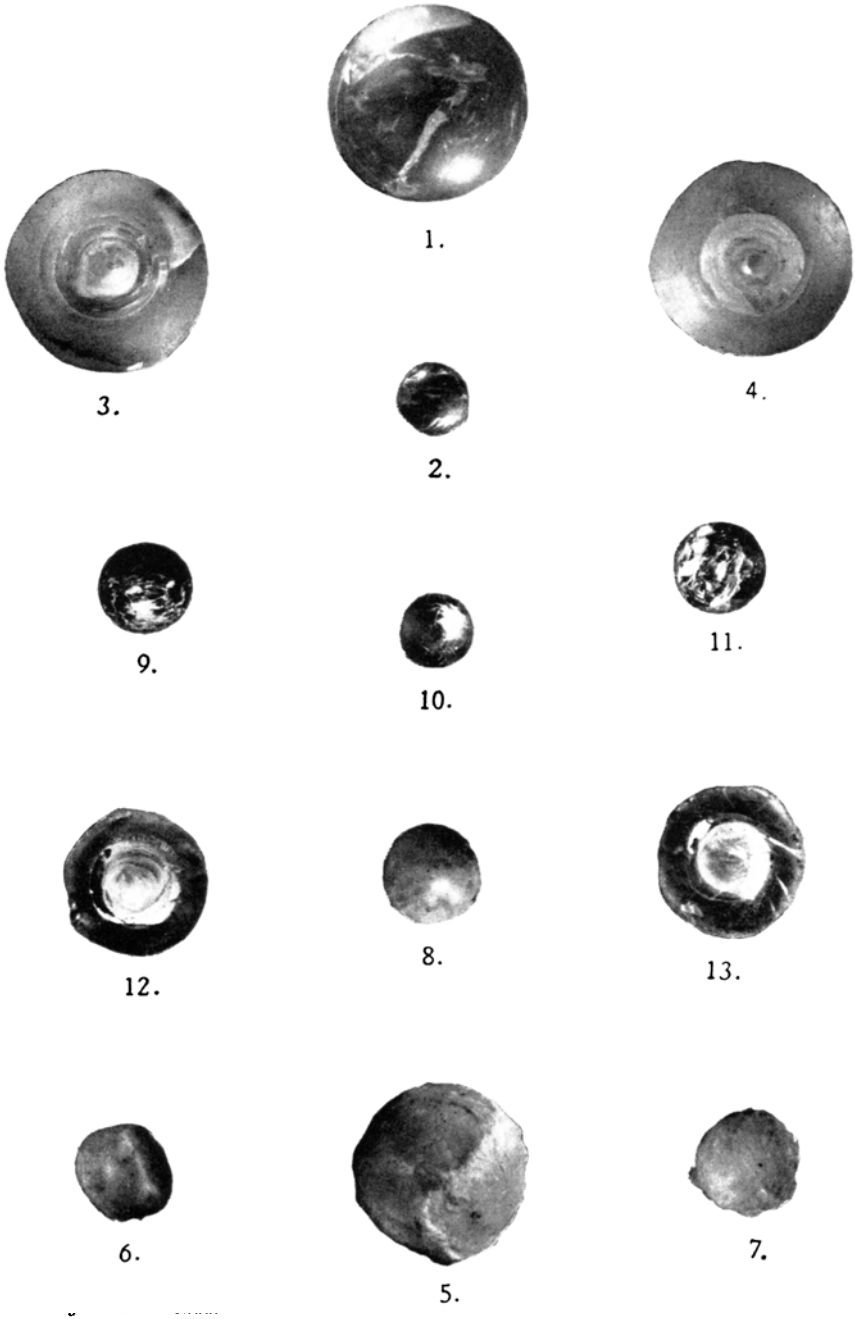

'Fish-pearls' from the Andamans.