CLVIII. THE OCCURRENCE OF VITAMIN D IN LAMPREYS (PETROMYZONTIDAE).

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ON the assumption that the storage of vitamin D in the livers of fishes is directly related to bone calcification, it might be expected that a decrease in the amount of vitamin D would be observed in passing from the Teleostei, or bony fishes, to the Elasmobranchii, or cartilaginous fishes, the skeleton of which contains very little calcium. The vitamin D storage should, on the same grounds, be still less in the Cyclostomata, a class of primitive vertebrates, distinct from fishes, in which the skeleton shows even smaller traces of calcification than that of elasmobranchs.

A number of species of Elasmobranchii has been investigated by Poulsson [1929, 1931] and by S. and S. Schmidt-Nielsen [1930], who established the presence of vitamin D in the livers, although in much smaller amount than in those of bony fishes, such as the cod. Poulsson suggested that the diffused ossification found in the vertebrae and the calcified deposits in the scales and teeth provided sufficient explanation of the presence of vitamin D. The vitamin D content of Cyclostomata has not previously been investigated by quantitative methods. Matzko [1929] examined one species, *Caspiomyzon wagneri*, which occurs in the Black Sea, in relation to its food value, and found that rats were protected from rickets by the large dose of 0.15 g. per day of the oil from the roasted flesh.

The principal subject of the present investigation has been the lampren, or river lamprey (*Petromyzon fluviatilis*), which is a fairly readily available species. In addition, a few specimens of the sea lamprey (*P. marinus*) have been examined. The liver oils, as well as those obtained from the ovary and from the eviscerated body of these two species, have been examined for vitamin D. The presence of vitamin A has also been investigated in view of the observations made by Mori [1904]. This author found that pills made of the lightly-cooked flesh of the sea lamprey were beneficial in the treatment of the infantile disease "hikan" (xerosis conjunctivae and keratomalacia) prevalent in Japan, although cod-liver oil was more efficacious. McCollum and Simmonds [1917] were the first to recognise this observation as an instance of the cure of vitamin A deficiency. The materials used by Mori have been referred to in the literature, through mistranslation, as "eel-fat" and "chicken-liver."

EXPERIMENTAL.

Material. The lampreys were caught in the River Severn at Tewkesbury in the months of March and April, 1930. At this season they ascend the river from the sea in order to spawn. Eighty-five specimens were received, mostly alive. Immediately after receipt the intestinal tract, liver, and gonads were removed. All the fish appeared to be sexually mature. The sea lampreys were caught in the same neighbourhood in May and June 1930, during the spawning migration. The season was a late and poor one. Only four specimens were received, all in good condition. We are indebted to Dr H. King of this laboratory for his assistance in obtaining these specimens through a local fisherman.

In order to compare elasmobranch fish with the Cyclostomata, some lesser spotted dogfish (*Seyllium canicula*) were examined. These were supplied by Plymouth Marine Biological Station in April and June, 1930. The females, received in April, were immature and the ovaries were rudimentary, but those received in June contained ova from 1 to 2 cm. in diameter.

Extraction of oil. Oils were obtained from the eviscerated bodies, livers, and ovaries of the lampreys, and from the livers of the dogfish. The same procedure was followed in all cases. The minced tissue was treated with 2 % aqueous potassium hydroxide solution (2 cc. to 1 g. of tissue) for 24 hrs. at room temperature, and then for 8–24 hrs. at 37°. After dilution with water, the solution was extracted with ether. Persistent emulsions were broken by the addition of a little alcohol. The extract was washed with water, dried over sodium sulphate, and the ether distilled off, finally under reduced pressure. The oils were stored in stoppered amber bottles in a refrigerator.

Determination of vitamin D. This was carried out by the method described by Bourdillon *et al.* [1931]. Rats rendered rachitic on Steenbock's rachitogenic diet were given comparable doses of the oils investigated and of a standard preparation of irradiated ergosterol, necessary dilutions being made in olive oil. X-ray photographs of the tibiae were taken at the beginning and end of the 14-day period of dosing and the degree of healing was determined by comparison with a calibrated radiographic scale. The antirachitic potencies are expressed in terms of the unit of the Medical Research Council, based on the standard solution issued by them.

Consideration of the "probable error" involved with the number of rats employed [*cf.* Bourdillon *et al.*, 1931] indicates that individual determinations may be subject to considerable error, but the figures obtained for a particular species, by summing the determinations on separate samples of the organs, are capable of indicating differences from another species with reasonable accuracy.

Determination of vitamin A. (a) *Biological measurements.* Owing to the small number of animals at our disposal and the limited quantity of most of the oils, only very rough and qualitative estimations of vitamin A contents

Table I. *Vitamin D determinations.*

Species	...	Lampren			Sea lamprey			Lesser spotted dogfish				
		Eviscerated body		Ova	Liver (i)		(ii)	Liver (i) (April)	(ii) (April)	(iii) (June)	(iv) (June)	(v) (June)
Tissue used												
Number (weight in g.) of fish used	♂ 11 (205) ♀ 13 (283)	30 (488) 19 (357)	38 (766) 12.6		2 (1505) 2 (1541)	1.9	13.8	3 (—)	3 (—)	2 (1780)	4 (3180)	1 (1093)
Proportion of tissue to whole fish (%)	87	1.8	10.6	11	3.0	1.2	26.4	104.4	155.3	68	103	62.2
Yield of oil, weight in g.	9.9	0.31	7.7	60	10.7	4.1	12.4	52	57	46	32	53.5
Yield of oil, % of tissue	8.3	3.8	23.1	15	23.7	50	102	3.7	7	8.3	5	9
Antirachitic potency of oil, M.R.C. units/cc.		53			44							
Antirachitic potency of tissue, M.R.C. units/g.	1.2	2.0	2.5	4.0	10.2	2.05	9.9	1.9	4.0	3.8	1.6	4.8
Number of pairs of rats used in each determination	4	3	7	3	2	3	3	5	3	3	3	3

Table II. *Vitamin A determinations.*

Species	...	Lampren				Sea lamprey				Lesser spotted dogfish				
		Evisce-rated body		Liver (i)	(ii)	(iii)	Ova	Sperm	Evisce-rated body	Liver (i)	(ii)	(iii)	(iv)	(v)
Tissue used														
Number (weight in g.) of fish used	♂ 41 (693) ♀ 32 (640)	17 (741) 19 (357)	30 (488) 19 (357)	38 (766) 12.6	7.6	80	2 (1505) 2 (1541)	2 (1541)	2 (1541)	2 (1541)	3 (—)	2 (—)	4 (—)	1 (1093)
Proportion of tissue to whole fish (%)	86	1.9	1.5	2.7	0.03	10.6	10.7	1.2	26.4	104.4	155.3	68	103	66.2
Yield of oil, weight in g.	40.5	0.51	0.28	3.9	0.3	11	6.0	23.7	4.1	12.4	52	46	32	53.5
Yield of oil, % of tissue	8.4	3.5	15	16	3.4	0	Very low	2.8	0.4	0.11	0.05	0.2	0.15	0.4
Blue value of oil	0.3	12.3	14.2	17.6	0.085	0	—	—	—	1.43	0.71	2.3	1.2	5.4
Blue value of tissue per g.	0.63	10.8	—	—	—	—	—	—	—	20	—	—	—	11
Vitamin A potency: cod-liver oil = 100	8	—	—	—	—	—	—	—	—	—	—	—	—	—
Number of rats used	2	—	—	2	—	—	—	—	—	2	—	—	—	3

of a few oils were made. Growing rats were kept on a diet deficient in vitamin A until loss in weight took place. The oils to be tested were then administered in daily doses of 100 mm.³ and cod-liver oil, for comparison, was given in doses of 20 mm.³, and the resumption of growth observed and measured over a period of 30 days. The relative potencies of the oils in the doses given were assumed to be proportional to the percentage increase in weight over this period, and the results are expressed as percentages of the potency of the cod-liver oil used, which had a blue value of 10.2.

(b) *Colorimetric measurements.* These are recorded, although we recognise the fact that a direct and quantitative relationship between the intensity of the antimony trichloride reaction and vitamin A content is not established. The blue values, referred to 40 mg. of oil, were determined with a Rosenheim-Schuster colorimeter by the method recommended by the sub-committee of the Pharmacopoeia Commission [1931], but determinations were sometimes made with undiluted oils.

The data of number and weight of fish, weight of tissues, and yield and potency of the oils obtained are given in detail in Tables I and II.

DISCUSSION OF RESULTS.

Comparison of the vitamin content of lampreys and other species.

The oils obtained from the body, liver, and ovary of both the lampern and the sea lamprey have been found to contain moderate amounts of vitamin D, and the antimony trichloride reaction, supplemented by a few biological tests, indicates that vitamin A is also generally present. The oil with the highest antirachitic potency was that from the ova of the sea lamprey, which was comparable with cod-liver oil. Only two specimens were examined, however, and the figures cannot be regarded as representative. The lesser spotted dogfish likewise yielded liver oils containing vitamins A and D, but in comparatively lower concentration. Since, however, different yields of oil are obtained from corresponding tissues of different species, it is preferable to base the comparison of species on the vitamin content calculated in terms of potency per g. of tissue; these figures are given in the eighth line of the Tables. The livers of lamperns, sea lampreys, and dogfish then appear to possess antirachitic potencies of roughly the same order. Even when account is taken of the relatively greater ratio of weight of liver to total body-weight in the dogfish, the vitamin D stored in its liver does not appear to be significantly greater than that stored in the livers of the lampreys. Moreover, lampreys contain, in addition, vitamin D in the body, which is rich in oil in comparison with that of the dogfish. The dogfish we examined yielded only 0.06 % of oil from the body, and sufficient was not obtained for determination of vitamin D.

Our results are, however, in agreement with those of previous workers who have shown that the livers of cartilaginous fish contain much less

vitamin D than those of bony fish such as the cod. The detailed and exact comparison of the vitamin content of different species is complicated by the influence of the factors of age, food, and sexual condition, as has been demonstrated in the case of the cod [Hess *et al.*, 1929; Drummond and Hilditch, 1930; Hawk, 1930], and it is our opinion that fuller attention must be paid to the variation due to these factors in each species, before definite conclusions can be drawn as to the variation of vitamin D content with species in relation to calcification, or other possible functions. The results we have obtained are based on a limited amount of material, and the evidence they yield must be used cautiously in considering the various problems involved in the calcium and fat metabolism of marine animals.

Relation of size of liver and oil content to sex. The proportion of weight of the liver to the total body weight in the female lampreys was about twice that in the male. The livers of the females were a translucent red, whilst those of the males were creamy and opaque. This difference in colour is probably due to difference in oil content, for the percentage yield of oil from female livers was about one-twelfth of that from male livers. The same difference in the yield of oil from the livers of the two sexes occurred in the sea lampreys. These constant differences in sexually mature lampreys of the same origin appear to be connected with the fat-storing function of the liver, and could perhaps be attributed to the greater transport of fat to the gonads which occurs in the female. Similar relations of size of liver and oil content to sex are known in the cases of the herring, salmon, and cod.

Distribution of vitamins and sex. Recently, investigations on cod have shown that the concentration of vitamin in the liver oil varies inversely with the amount of oil in the liver [Hess *et al.*, 1928, 1929; Drummond and Hilditch, 1930], from which the conclusion has been drawn that vitamins are not transferred to the gonads proportionately with the fat, but to a lesser degree. It might have been expected that comparison of sexually mature fish of different sexes would have shown some striking differences in the vitamin potencies of the liver oils, if it were further assumed that there had been a great difference in the transport of the fat to the gonads in the two sexes. Analysis of the data we have obtained shows, in fact, no evidence of considerable differences of this kind in the contents of either vitamin A or D.

Significance of the presence of vitamin D in lampreys. It is possible to argue that the presence of vitamin D in lampreys and elasmobranch fish is entirely fortuitous, and that it is absorbed from the foods with the fats and accompanies the latter in their distribution in the body. It seems much more attractive to suppose, however, that the vitamin is of physiological importance. Its function might then be explained by the presence of partly calcified structures. The cartilage of the head, for instance, is very hard, and we have been able to demonstrate qualitatively the presence of calcium in the horny teeth of *P. marinus*.

SUMMARY.

The oils extracted from the eviscerated body, liver and ovary of the lampern (*Petromyzon fluviatilis*) and the sea lamprey (*P. marinus*) have been found to contain vitamin D in moderate amount. Data for comparison with a typical cartilaginous fish have been obtained by examination of the liver oil from the lesser spotted dogfish (*Scyllium canicula*). The results indicate that there is little difference between the antirachitic potencies of the tissues of the species examined, although the antirachitic potencies of the livers are much lower than those of the cod. The bearing of the results on a possible correlation between vitamin D content and extent of calcification and on the transference of vitamin D in the body have been considered. Vitamin A is also found to be present in the oils examined.

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