XXXIV. THE VITAMIN A CONTENT OF AUSTRALASIAN FISH LIVER OILS. I

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The great majority of the species of fish round the shores of Australia and New Zealand are unknown to the northern hemisphere, and consequently few scientific data are available with regard to their value as food. Practically nothing is recorded of the liver oils of the local fish, and an estimation has been made of the vitamin A contents of the liver oils of some varieties of fish which abound in or about Victoria. In chronological order of investigation the fish examined are the Australian salmon (*Arripis trutta*), snapper (*Pagrosomus auratus*), Murray cod (*Oligorus macquariensis*), school or snapper shark (*Galeorhinus australis*), kingfish or yellowtail (*Seriola grandis*), blue-fin tuna or southern tunny (*Thynnus maccoyi*), black bream (*Sparus australis*) and barracouta (*Thyrsites atun*).

The estimation of vitamin A has been carried out by non-biological methods, namely (*a*) the Carr-Price method, using the Lovibond tintometer to estimate the intensity of the blue colour, and (*b*) measurement of the intensity of absorption at 328 mμ of a solution in *cyclo*hexane, using the Hilger Vitameter AC. In all cases the estimation has been done on the non-saponifiable portion of the liver oil. We are aware of the advisability in some cases of a biological check on the spectrographic and Carr-Price methods [Ward & Haines, 1936; Anonymous, 1936], but there is at present no means of carrying out such a biological assay in Australia. However, by utilizing the practical experience gained by one of us in the non-biological methods used in Prof. Drummond's laboratory in London, and by standardizing the apparatus with halibut oil of known value, trustworthy results have been obtained.

Fair agreement is obtainable between the Carr-Price and the spectroscopic methods. A serious source of error in the use of the vitameter is due to the "flickering" which is soon apt to take place in the arc. To reduce these changes in intensity to a minimum, it is advisable in the final and most accurate determination to have the two copper rods ending in sharp points. The point of the vertical rod is exactly over the pointed end of the horizontal rod, and the two ends are about 1–2 mm. apart. It is true that the sharp ends require much more frequent grinding than when blunt ends are used, but their use gives concordant results. This source of error was not discovered in the July estimation, but all the figures obtained are nevertheless included. The values obtained are expressed as percentages of vitamin A, using the results of Carr & Jewell [1933].

Experimental

Extraction of oil from liver. Steaming of the minced liver was first tried, but no satisfactory separation of the oil was obtained. The procedure finally adopted was as follows.

The livers were counted, minced and weighed; then treated with about 2/3 wt. of Na_2SO_4 anhyd. following the procedure of Lovern *et al.* [1933], and left in the desiccator overnight. The dry mass was then ground up and refluxed with light petroleum (b.p. 60–80°) for about 6 hours. The solution was dried with

Na_2SO_4 and the ether distilled off, finally *in vacuo*. A sample of the oil was then saponified and the estimations were carried out on the "sterol-free" non-saponifiable fraction.

Saponification of oil. The process followed was that recommended by the International Conference on Vitamin Standardization [1934]. A slight variation was introduced, in that the sterols were crystallized from a solution of the non-saponifiable fraction in pure methyl alcohol, the alcohol removed *in vacuo*, and the estimation carried out.

In certain later cases, when the amount of oil available was very small, this variation was dispensed with. The non-saponifiable fraction was made up directly in chloroform (the last traces of ether could not be removed *in vacuo*).

Iodine values. A determination of the iodine value was made on each of the oils.

The method used was that of Dam [1924]. A solution of the oil in $CHCl_3$ is treated with at least 40% excess of the pyridine sulphate-bromine reagent.

The system is kept in the dark for 15 min., water and KI are added and the excess of brominating agent determined by titration with $Na_2S_2O_3$. At the same time a blank test is made with the same quantities of reagents and $CHCl_3$.

School shark. This oil proved by far the most potent of those examined and was treated in somewhat greater detail. In addition to the usual properties, the saponification value was determined.

In this case, too, the extremely high oil content permitted satisfactory extraction of the oil by steaming. Hence oil was extracted by steaming and desiccation separately, and the two compared.

Results

Fish	Date obtained 1936	No. of livers	Average wt. g.	Yield oil %	"Sterol-free" non-sap. %	Iodine value	Sap. value	Vitamin A: Carr-Price %	Vitamin A: Vitameter %
Salmon	10. vii	About 70	—	7·6	1·1	96	—	0·089	0·116
,,	15. vii	130	11·2	7·3	1·9	97	—	0·063	0·077
,,	19. xi	69	10·8	3·2	2·5	105	—	0·21	0·22
,,	19. xi	2	16	6·8	—	—	—	0·27	—
,,	19. xi	3	7·3	3·1	—	—	—	0·13	—
Snapper	15. vii	21	68·0	Some lost	1·3	99	—	0·103	0·101
,,	24. vii	10	80	5·8	4·4	84	—	0·038	0·034
,,	6. xi	12	83	4·9	2·0	109	—	0·33	0·31
Murray cod	25. viii	12	33	6·2	0·07	107	—	0·07	0·066
School shark (Na_2SO_4)	28. viii	2	575	49·9	2·7	157	175	0·91	0·85
School shark (steaming)	28. viii	14	467	30	2·4	161	172	0·99	0·98
Yellow tail	13. x	1	247	7·3	1·8	92	—	0·031	0·040
,,	16. x	11	236	10·9	1·9	137	—	0·46	0·44
Blue-fin tuna	13. x	1	148	2·0	—	109	—	0·18	0·20
Bream	19. xi	1	30	5·2	—	111	—	0·61	—
Barracouta	11. xii	About 65	11·1	1·8	6·3	160	—	2·3	2·4

Note on yield of non-saponifiable fraction. Where no figure is given, the estimation was carried out without removal of the sterols.

In the second batch of snapper oil, the non-saponifiable fraction contained solid, instead of an oil, and the high figure is probably due to residual sterols.

A comparison of this table of results with one dealing with common fish of the northern hemisphere [Lovern *et al.*, 1933], shows that if the halibut is excluded, the vitamin A contents of the Australian fish liver oils are definitely

higher. If the halibut is neglected, only 5 out of some 22 northern hemisphere fish have liver oils containing 0·1 % or more of vitamin A. Out of the 8 Victorian fish examined all except the Murray cod (which is the only fresh-water fish in the list, and which has been examined only at the beginning of spring) average over 0·1 % vitamin A. An examination of a larger variety of Australasian fish is desirable if only from this point of view.

Lovern *et al.* have shown that in halibut liver oil there is an increase of vitamin A content in early summer as opposed to winter. Though a detailed study of this point has not been made, the analyses of Australian salmon and snapper in July and November and December shows that there is a similar seasonal change in the local fish, the November figures in all cases being higher than the July values of vitamin A. The figures in the table of results also lend themselves to other generalizations, such as the relations between iodine value and vitamin A content, but these will be discussed when a greater variety of fish has been studied.

Though several of the local fish are seen to have liver oils richer in vitamin A than most cod liver oils, only 1 (with the possible exception of the Australian salmon which frequents the coast in vast shoals which could be easily netted) out of the 8 examined can be claimed to constitute a discovery of economic importance. The school or snapper shark is caught in large quantities by line when fishing for snapper etc., and after the more prominent shark features have been removed it is widely sold and eaten, though not under its proper name. The fish is very common, grows to a length of more than 5 ft. and has a liver averaging over 1 lb. in weight. Only the early spring (August) shark has so far been examined, and there is a great probability that the early summer livers will have a much higher content of vitamin A, judging by the fact that the livers of the prey of the shark have a definite seasonal increase. In any event, though the oil with its content of about 1 % of vitamin A is perhaps less rich than the liver oil of most halibuts, it must be borne in mind that the liver of the school shark contains about twice as much oil as the liver of the halibut. The shark livers steamed give always more than 30 % of oil separated by simple decantation, and when a method is used in which all the oil is extracted, the yield is about 50 %. (Incidentally it is noteworthy that stearin seems to settle out more easily when the livers are steamed than when they are extracted with light petroleum.) The halibut liver [Lovern *et al.*, 1933] averages about 12–35 % of oil, approximately half the content of the shark liver.

The school shark liver oil is worthy of more detailed investigation. It can be readily distilled, but the relatively low iodine value (157–161) and the small amount (2·4–2·7 %) of non-saponifiable matter (of which over one-third is vitamin A) show that little if any squalene can be present, and that it is likely that the oil can be assimilated as well as many other oils which are widely used as food.

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