XL. THE DISTRIBUTION OF VITAMINS A AND A_2. II

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In the present phase of research on the A-vitamins two facts merit special attention. One concerns the distribution of the vitamins in respect of the absolute amounts found at different sites in different species and the other the relative proportions of the two vitamins.

The first fact is that a great deal of vitamin occurs in the absorptive parts of the alimentary tracts of many, but not all, species of fish. The second is that vitamin A_2, which is practically confined to fishes, shows in all species a distribution over the body which differs appreciably from that of vitamin A. Vitamin A_2 predominates over vitamin A only in fresh water fishes; it has, however, been detected in birds subsisting on fish from inland waters.

Earlier work [Edisbury *et al.* 1938] on the distribution of the two vitamins has been extended.

Experimental

Sturgeon (Acipenser sturio)

A large sturgeon 10 ft. 6 in. long was landed at Aberdeen in May, 1938. The viscera were obtained and extracted in the laboratory.

The pyloric caeca, which form a compact mass in the sturgeon, were missing and had probably been thrown away by the fish merchant. The stomach was not freed from adhering mesentery but showed a high oil content which could not be attributed to mesenteric fat.

Table I

	Wt. g.	Oil %	Wt. of oil g.	Vitamin in oil %	Wt. vitamin g.	Vitamin A p.p.m. in tissue
Liver	3941	37·2	1466	1	14·66	*c.* 3000
Intestines*	1541	1·9	29·3	10	2·93	1900
Stomach	233	25·7	60	0·01	0·006	26
Spleen	145	4·8	6·96	0·015	0·001	7
Mesentery	75	24·0	18	0·006	0·0011	14

* Minus pyloric caeca.

Table II. *Spectroscopic tests on oils*

		Liver oil	Intestinal oil	Spleen oil	Stomach oil	Mesenteric oil
$E^{1\%}_{1\,cm.}$ 693 mμ	Colour test $SbCl_3$	11	40	Weak	Not detectable	Not detectable
617 ,,		47	509	0·36	0·18 (600 mμ)	0·12 (600 mμ)
583 ,,		26	272	0·38	0·23 (572 mμ)	0·16 (572 mμ)
325 ,,	Direct absorption	17	157	—	—	—
		No inhibition of colour test		Marked inhibition of colour test with $SbCl_3$		
Ratio $E^{1\%}_{1\,cm.}$ 617/693 mμ		4·3	12·7			

This large sturgeon contained some 30 g. of vitamin A esters and perhaps 7 g. of vitamin A_2 esters in its liver. The complete intestines must have contained at least 6 g. of vitamin A esters and perhaps 0·5 g. of vitamin A_2 esters. These quantities are large for a single fish and support the possibility previously suggested that the A-vitamins possess functions not hitherto recognized.

Possibly vitamin A_2 is passed on to the liver from the intestines more readily than vitamin A, since the ratio A/A_2 is much higher in the intestines than in the liver. This is also true of salmon, trout and halibut [Edisbury *et al.* 1938]. The distribution of the two vitamins suggests that they may not exert identical functions at all sites.

Lampern (Petromyzon fluviatilis)

In order to illustrate the kind of contrast which has already been noticed between, for example, eels [Edisbury *et al.* 1937] and halibut [Edisbury *et al.* 1938], the sturgeon may be compared with the freshwater lamprey. Three sets of data were obtained and gave results in fair agreement.

Lamperns (Worcester), Nov. 1937. 6♂, 6♀. Total wt. 439 g. Average wt. 36·6 g.

	Digestive glands	Fore-gut	Hind-gut	Gonads and excretory glands	Eyes	Bodies: Head and thorax	Bodies: Abdomen and tail
Wt. g. ...	6·45	1·4	1·28	35·4	1·0	109·5	284·5
$E^{1\%}_{1\,cm.}$ 693 mμ	Not seen	Not seen	0·16	0·13	—	0·01	0·007
617 „	0·185	0·37	1·7	1·18	0·015	0·03	0·016
583 „	0·12	0·2	0·9	0·6	—	0·023	0·012
325 „	—	—	—	—	—	0·027	0·016
Approx. wt. of vitamin A, mg.	0·24	0·1	0·4	8·26	0·003	0·04	0·08
Vitamin A, p.p.m.	37	70	312	233	3	0·37	0·38

It is noteworthy that vitamin A predominates over vitamin A_2 and is very widely distributed in small amounts.

Dogfish (Squalus acanthias)

Some species are noteworthy for possessing abnormally low vitamin A reserves in the liver. Our experience of dogfish liver oil is that it commonly contains very little vitamin but it would be rash to generalize because we have examined one specimen (from Newfoundland) which was unusually rich. If a species tends to store little vitamin A in the liver, the present work makes it interesting to examine its intestines. We therefore retained a quantity of such material from dogfish.

558 g. of dogfish intestines were decomposed by means of alcoholic potash; 2·25 g. of non-saponifiable extract, obviously mainly sterol, were obtained. This material gave the following tests:

$$E^{1\%}_{1\,cm.}\begin{cases}693\ m\mu & 0{\cdot}46\\ 617\ \ ,, & 8{\cdot}5\\ 580\ \ ,, & 5{\cdot}27\end{cases}$$

The vitamin A content of the "non-sap." is of the order 0·2 % so that the intestines contain:

c. 4000 p.p.m. of sterol,
8 p.p.m. of vitamin A,
0·3 p.p.m. of vitamin A_2.

The contrast with halibut intestines containing up to 35,000 p.p.m. vitamin A is very striking and suggests a radical difference between the two species in respect of the mechanism of fat absorption (see Part III in this *Journal*, p. 330).

We have already noted [Edisbury *et al.* 1938] a marked decrease in the vitamin A content of the intestines of halibut as the absorptive function decreases in importance towards the vent. This has been confirmed. Thus the entire rectal caecum of one fish yielded 0·8 % of oil containing 4·25 % of vitamin, corresponding to 340 p.p.m., as against 33,000 p.p.m. for the absorptive parts of the gut.

The stomach of halibut is likewise poor in oil. The entire stomach of one fish yielded 0·5 % of oil, 26·7 % of which was non-saponifiable matter, mainly cholesterol. The vitamin A content was of the order 2 p.p.m. The stomach "oil", if dissolved in a suitable solvent to saturation at the boiling point, yields a well crystallized mixture of sterol and sterol esters on cooling. A halibut caught in Sept. 1938 was obtained with viscera in a good state of preservation. The stomach tissue was split up into various coats (see Part III in this *Journal*, p. 330) and the amount of non-saponifiable matter was determined:

	Wt. g.	Non-sap. p.p.m.
Layer 1 (inside)	214	3900
2	596	3200
3	263	4150
4	562	3070

The proportion of sterol did not exceed that usual in muscle cells, and the lining of the stomach contained little or no fat and practically no vitamin A.

The sterols from the various layers were recrystallized and some were obtained pure enough to warrant quantitative determinations of provitamin D content (probably 7-dehydrocholesterol). Cholesterol from cod liver oil, salmon liver oil and similar sources shows the ergosterol-7-dehydrocholesterol absorption bands at 293·5, 281·5 and 270 mμ, $E^{1\%}_{1\,\text{cm.}}$ 281·5 mμ 0·1 to 0·3 corresponding to some 0·03–0·1 % of provitamin. The sterol from halibut stomach also shows these bands but at much higher intensity, $E^{1\%}_{1\,\text{cm.}}$ 281·5 mμ 6·2 and 4·5. No explanation of this has been found, but it is possible that the provitamin D is a product of dehydrogenation of cholesterol occurring in the tissue.

Halibut spleen from the same fish was also studied. It yielded *c.* 5300 p.p.m. of non-saponifiable matter, *c.* 380 p.p.m. of vitamin A and *c.* 35 p.p.m. of vitamin A_2. Recrystallization of the non-saponifiable matter from methyl alcohol gave sterol in good yield. One further recrystallization gave a snow-white sample of cholesterol and an almost colourless mother liquor. Spectroscopic examination of both sterol and mother liquor revealed highly selective absorption of an unexpected type. The broad persistent absorption band with λ_{max} 275 mμ showed no sign of resolution into the narrow bands characteristic of ergosterol and dehydrocholesterol. The intensity of absorption, $E^{1\%}_{1\,\text{cm.}}$ 275 mμ 4·6, in the recrystallized sterol was by no means negligible and perhaps indicates that the cholesterol was contaminated with 1–2 % of a substance not so far identifiable.

Similar experiments on bullock spleen and sheep spleen failed to show the presence of this absorbing impurity in mammalian spleen.

Bullock spleen:

Non-sap. 5200 p.p.m.
Carotene *c.* 1·5 p.p.m.

Recrystallized sterol showed three bands corresponding with those of ergosterol and 7-dehydrocholesterol (0·02 % in the cholesterol).

Sheep spleen:

Non-sap. 4800 p.p.m.

Carotene 0·26 p.p.m.

Recrystallized sterol contained *c.* 0·06% of "dehydrocholesterol". The amount of vitamin A in the bullock and sheep spleens was extremely minute.

Vitamin A in sea-birds

In view of the considerable, though variable, amounts of vitamin A occurring in the absorptive parts of the intestines of fishes, it seemed desirable to study its distribution in birds subsisting mainly on fish. The herbivorous mammal does not retain more than a few parts per million of vitamin A in its intestines. The following data must be regarded as preliminary to further work. The figures indicate, however, the presence of appreciable amounts of vitamin in the intestines.

Herring gull (Larus argentatus)

The liver (29 g.) yielded 0·172 g. of non-saponifiable matter, 23% of which (0·04 g.) consisted of vitamin A corresponding to 1380 p.p.m. in the tissue. Vitamin A_2 could not be detected.

The intestines (43 g.) yielded 0·194 g. of non-saponifiable matter containing 2·9% (0·0056 g.) of vitamin A or 130 p.p.m. Vitamin A_2 was again absent.

The heart and lungs both gave extremely low values (<1 p.p.m.) for vitamin A.

Skua gull (Megalestris catarrhactes)

The livers from 8 birds weighed 300 g. and yielded 2·2 g. of non-saponifiable extract containing 32% (0·7 g.) of vitamin A. This corresponds to *c.* 2300 p.p.m.

The intestines (345 g.) contained 1·8 g. of non-saponifiable matter, 4% of which was vitamin A. This is equal to 208 p.p.m. of vitamin.

The hearts, eyes and lungs all contained less than 1 p.p.m. vitamin A. No vitamin A_2 was detected in any part of the birds.

Gannet (Sula bassana)

One bird was dissected. The lungs were very large (74 g.) and gave 0·341 g. of non-saponifiable extract, 19% (0·065 g.) of which was vitamin A. This corresponds to 870 p.p.m.

The liver (5·5 g.) was abnormally small and only contained a few p.p.m. of vitamin A.

The intestines (54 g.) yielded 0·231 g. of non-saponifiable matter mainly sterol and the vitamin A content did not exceed 10 p.p.m. The heart (30·5 g.) appeared to contain 0·7 p.p.m. vitamin A.

If these results are to be believed, the lungs take precedence over the liver and intestines as sites for vitamin A deposits in this species. The data are very surprising but we have found no valid reason for their rejection. A number of gannets will be studied as they become available.

Summary

A large sturgeon yielded 1·47 kg. of liver oil containing 1% of vitamin A, whilst part of the intestines gave 29·3 g. of oil containing 10% of vitamin. The liver contained *c.* 30 g. vitamin A esters and 7 g. vitamin A_2 esters. The intestines probably contained 6 g. and 0·5 g. of vitamin A and vitamin A_2 esters

respectively. The ratio vitamin A/A_2 was much higher in the intestines than in the liver.

Lamperns are not rich in vitamin A but small quantities occur at a number of sites. Vitamin A predominates over vitamin A_2.

The dogfish, which yields a liver oil poor in vitamin A, also possesses extremely little in its intestines. Those parts of the alimentary tract of the halibut which take little part in the assimilation of fat are likewise poor in vitamin and it may be that the dogfish and some other species do not use vitamin A in absorption to the same extent as the halibut, cod and salmon.

A beginning has been made with the study of the distribution of provitamin D in the cholesterols obtained from different organs. The non-saponifiable fraction of spleen is noteworthy for the occurrence of small quantities of a substance with λ_{max} 275 mμ. This substance has not so far been detected in mammalian spleens.

Sea birds (herring gull and skua) have much more vitamin A in their intestines than such animals as the rabbit. The case of the gannet is surprising in that its lungs contain relatively large quantities of vitamin.

The work as a whole leads to the conclusion that vitamins A and A_2 probably do not replace one another with equal readiness in all functions. The enormous variations in the vitamin A content of fishes' intestines make it probable that the mechanism of assimilation is different when vitamin A is a major intestinal constituent from that obtaining when the vitamin is a trace constituent.

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REFERENCES

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