LVIII. DISTRIBUTION OF VITAMIN A IN THE TISSUES OF THE EELS *ANGUILLA VULGARIS* AND *A. AUCKLANDI* RICH.¹

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(Received 27 January 1937)

It is usual to regard the liver as the only considerable depot for vitamin A in the vertebrate body. Numerous instances are recorded of vitamin A or carotenoid pro-vitamins occurring in appreciable quantities in a variety of tissues—the M.R.C. Special Report on vitamins [1932] included a comprehensive list which has since been amplified—but the general impression gained from the literature is that the total amount of vitamin in non-hepatic tissues is always unimportant in comparison with the liver reserves. Perhaps as a result of this feeling, relatively little quantitative study has been accorded to oils from other parts of the body, and in any event the majority of such oils give little or no blue colour with antimony trichloride, either before or after saponification. Few exceptions have been reported. The present authors found spectroscopic evidence of vitamin A in conger eel peritoneal fat and in the peritoneal and pancreatic fats of the sturgeon [Edisbury *et al.*, 1933], but there seemed at the time no reason to suppose that in some species other body tissues might contain a total quantity of vitamin A equal to, or even exceeding, the liver reserves, despite the necessarily far lower concentration in the body.

In the present work, two species of eel have been studied:

- (i) *Anguilla vulgaris*, from Holland and the Dee Estuary, Scotland, and
- (ii) *Anguilla aucklandi* Rich., from New Zealand, by courtesy of the Dominion Government Authorities who supplied about half a ton of fish, transported in cold storage. (This eel is also variously termed *A. aucklandii* and *A. dieffenbachii*. According to Phillipps [1932] the latter is the more strictly correct designation on grounds of priority; but *A. aucklandi* appears to have the widest usage.)

The preliminary experiments were concerned only with determining the potencies of the liver oils from specimens of different sizes [cf. Lovern *et al.*, 1933; MacPherson, 1932; 1933; 1934]. Some compared favourably with halibut liver oil in vitamin A content, but the percentage of oil yielded by the liver was very much lower than we had previously encountered in any species of fish. These "oils" were solid at room temperature, most melted at body temperature, but some remained highly viscous up to 100°; all were gummy materials resembling crude concentrates in appearance and had a curious odour reminiscent of vegetable unsaponifiable matter. The percentage of unsaponifiable matter was determined for some of the oils and found to be 16–18 %.

¹ Part of this paper was read at the 179th Meeting of the Biochemical Society; cf. *J. Soc. chem. Ind., Lond.*, 55, 508 (1936).

Later experiments included the examination of oils from head, skin, body (musculature and skeleton, excluding skull), liver and the remainder of the viscera; whilst, later still, bodies were divided at the vent into roughly equal trunk and tail portions which received separate treatment. The non-liver oils were in general much paler in colour than the liver oils, melted more freely and had a normal odour. Although individually reproducible, the yields obtainable from corresponding tissues varied considerably between different batches of eels.

The estimates of vitamin A content recorded in the ensuing tables have all been determined spectroscopically on the ether-extracted oils, which are assumed to contain the whole of the vitamin originally present in the tissues. Weak oils were saponified following our standard technique [Hume & Chick, 1935, p. 52; also Morgan *et al.*, 1935] and the blue colour with antimony trichloride was studied using a visual spectrophotometer. Some of the richer materials were assayed by the ultraviolet absorption method, taking the intensity of absorption near $328\text{ m}\mu$ (actually $325\text{ m}\mu$ for most of these eel products) as the criterion. This method was, however, found unsuitable for certain of the materials owing to the presence of absorption of an irrelevant nature which was exhibited even by the unsaponifiable extracts and could not always be eliminated. In such circumstances—fortunately rare, excepting in very weak materials such as herring oil, haddock liver oil, blood—the blue colour test is regarded as the most satisfactory compromise and has accordingly been used exclusively in some series. Calculation of the percentage of vitamin A is on the basis of $E_{1\text{ cm.}}^{1\%}$ 620 $\text{m}\mu$, 5000; 583 $\text{m}\mu$, 2600 (colour test); 328 $\text{m}\mu$, 1600 (ultraviolet); Carr-Price value 78,000, obtained for the richest concentrate examined by us [Carr & Jewell, 1933], which is arbitrarily taken as 100% vitamin A and which, it is believed, was not far from "pure" $\text{C}_{20}\text{H}_{29}\text{OH}$. In the absence of irrelevant absorption in the ultraviolet and of inhibitors in the colour test (which affect the 620 $\text{m}\mu$ band preferentially and must be allowed for as far as possible, together with a direct experimental error of $\pm 5\%$, in arriving at an estimate) the amount of vitamin A is directly proportional to the intensity of absorption at the maxima specified. Owing to the difficulties encountered in the present investigation the limits of error are likely to be rather wider than usual, but they probably do not exceed $\pm 10\%$. All the figures recorded here must be regarded as referring only to the $\text{C}_{20}\text{H}_{29}\text{OH}$ content. No assumptions are made concerning possible differences in the biological activity of isomerides etc., and any hypothetical transparent and/or

Table I. *Vitamin A content of livers from Anguilla aucklandi*

Weight of liver	Oil content of liver	Carr-Price	Vit. A content of oil from B.U.	$E_{1\text{ cm.}}^{1\%}$ 328 $\text{m}\mu$ (u.v.)	Vit. A content of oil from u.v.	Vit. A content of whole liver tissue mg./100 g.	Approx. total vit. A per liver mg.
g.	%	B.U.	%		%		
—	3	2600	3.3	56	3.5	105	—
7.1	1.6	640	0.82	13	0.81	13	0.92
19.5	1.2	3180	4.07	58.3	3.64	44	8.5
<5	3.0	1620	2.08	31.5	1.95	59	Up to 2.9
5-7	1.5	1660	2.13	34.3	2.15	32	1.6-2.2
7-12	2.1	1480	1.9	35.5	2.22	47	3.2-5.6
12-15	1.4	1560	2.0	44	2.75	39	4.6-5.8
15-20	2.1	[1000]	—	34.3	2.15	45	6.7-9.0
20-25	2.5	1900	2.44	43	2.7	67	13.5-16.9
25-30	1.4	2490	3.19	55	3.44	48	12.0-14.4
>30	1.6	1860	2.38	47	2.93	47	Over 14

Table II. *Vitamin A content of livers from Anguilla vulgaris*

Average weight of livers, g.	No. of eels in batch	Oil content of livers %	Carr-Price B.U.	Vit. A content of oil from B.U. mg./100 g.	Spectroscopic colour test		Vit. A content of oil from colour test mg./100 g.	Vit. A content of whole liver tissue mg./100 g.	Average total vit. A per liver mg.
					$E_{1\text{cm.}}^{1\%}$ 620 m μ	583 m μ			
0.53	226	2.1	160	200	11.2	6.4	220 240 } 230	4.8	0.025
0.86	236	2.1	200	250	12.0	7.83	240 310 } 300	6.3	0.054
1.41	132	2.05	330	420	17.5	10.9	350 420 } 400	8.2	0.12
2.09	52	2.3	410	530	20.6	12.0	410 460 } 450	10.4	0.22
2.70	67	2.9	380	480	24.0	13.5	480 520 } 500	14.5	0.39
4.55	27	2.9	385	490	17.5	11.0	350 420 } 400	11.6	0.53
12.5	5	4.1	560	720	32.5	17.4	650 670 } 660	27.1	3.4

Note. On an average, these livers represent 3.2% of the weight of the entire eel.

Table III. *Vitamin A content of the largest eels in Table II (average weight 391 g.)*

	Percentage of total wt. of eel	Oil content %	Vit. A content of oil mg./100 g.	Vit. A content of original tissue mg./100 g.	Total vit. A in tissue mg.
Liver	3.2	4.1	660	27	3.4
Viscera	3.0	8	20	1.6	0.2
Body	93.8	14	12	1.7	6.2

Table IVa. *A. aucklandi. Total quantity (mg.) and concentration (mg./100 g.) of vitamin A in tissues and oils*

Av. wt. of eels kg.	No. of eels in batch	Body			Liver			Viscera			Entire eel	
		Whole tissue			Whole tissue			Whole tissue			Whole tissue	
		Oil mg./100 g.	mg./100 g.	mg.	Oil mg./100 g.	mg./100 g.	mg.	Oil mg./100 g.	mg./100 g.	mg.	mg./100 g.	mg.
0.37	11	4	0.26	0.84	540	36	1.5	15	0.48	0.06	0.64	2.4
0.50	10	10	0.74	3.5	970	62	3.4	42	0.97	0.19	1.4	7.1
0.79	11	8	0.77	5.7	690	45	3.8	26	0.68	0.22	1.2	9.7
1.2	13	8	1.0	11.5	550	41	6.5	11	0.51	0.32	1.5	18.3
1.6	10	10	1.8	26	810	66	13	18	0.65	0.54	2.5	40
2.0	6	14	3.1	56	920	62	15	25	1.15	1.25	3.6	72
3.0	11	12	2.5	68	820	58	22	20	1.42	2.4	3.1	92
3.9*	1	29	1.5	56	4100	123	64	ca. 30	ca. 0.3	ca. 0.45	3.1	120

* One specimen only. Very low fat content. Cf. Table IVb.

Table IV*b*. *A. aucklandi*

Average weight kg.	Average length cm.	Body		Liver		Viscera	
		Oil content %	Oil content %	Percentage of total weight of eel	Percentage of total weight of eel	Oil content %	Percentage of total weight of eel
0.37	48	6.5	6.7	1.11	3.2	3.46	3.82
0.50	53	7.4	6.4	1.08	2.3	4.11	5.25
0.79	63	9.6	6.5	1.06	2.6	5.18	5.45
1.2	74	12.7	7.4	1.33	4.6	5.64	3.74
1.6	81	17.8	8.1	1.24	3.6		
2.0	86	21.9	6.8	1.23	4.6		
3.0	94	20.7	7.1	1.25	7.1		
3.9	107	5.2	3.0	1.33	1.1		

Table V. *A. aucklandi* (*tissues other than liver or viscera*)

	Wt. of tissue g.	Oil content %	Vit. A content of oil mg./100 g.	Vit. A content of whole tissue mg./100 g.	Wt. of vit. A mg.	Total non-liver vit. A mg.	Non-liver vit. A content of whole eel mg./100 g.
(1) Length of eel 84 cm.; weight 1.35 kg.							
Body tissues:							
Trunk	472	3.5	20	0.7	3.3	17.4	1.29
Tail	416	17.3	17	2.9	12.2		
Head	252	3.8	9.7	0.37	0.93		
Skin	140	22.1	3	0.66	0.93		
(2) Length of eel, 89 cm.; weight 1.9 kg.							
Body tissues:							
Trunk	663	9.9	15	1.5	9.9	24.9	1.31
Tail	733	19.4	9	1.7	12.8		
Head	107	6.5	8.3	0.54	0.58		
Skin	238	34.9	2	0.7	1.65		

We are unable to account for the very low oil content of the trunk (i.e. the fore portion of the body, including the visceral cavity) of the smaller eel, or for the apparent inversion of head weights. The latter phenomenon has repeatedly been observed, despite a standard procedure in severing the heads from the bodies.

Table VI. *A. vulgaris*

	Weight of tissue g.	Oil content %	Vit. A content of oil mg./100 g.	Vit. A content of whole tissue mg./ 100 g.	Weight of vit. A mg.	Total non- liver vit. A mg.	Non- liver vit. A mg./ 100 g.	Total vit. A mg./ 100 g.
Group 1. Average length of eels (9) 86 cm.; weight 1.26 kg.								
Body tissues:								
Trunk	472.5	24.7	13	3.2	15.2	34.5	2.7	3.8
Tail	351.1	29.1	11	3.2	11.2			
Head	92.0	10.6	8.5	0.9	0.83			
Skin	290.0	22.7	9.5	2.16	6.25			
Viscera	45.5	13.9	17	2.35	1.07			
Liver	16.0	8.4	1050	88	14.1			
Group 2. Average length of eels (8) 96 cm.; weight 1.53 kg.								
Body tissues:								
Trunk	571.4	25.0	19	4.75	27.1	64.5	4.2	6.9
Tail	462.4	25.8	21	5.42	25.1			
Head	106.1	15.0	9	1.35	1.4			
Skin	325.5	25.9	11	2.85	9.3			
Viscera	43.5	15.2	25	3.8	1.65			
Liver	24.5	20.5	800	164	40.2			
Percentage of total weight								
			Viscera		Liver		Percentage of total vitamin A in	
Group 1			3.61		1.25		Head	Skin
Group 2			2.84		1.61		1.7	12.8
							1.3	8.8

Table VII. *Distribution of total vitamin A*

Av. wt. of eels kg.	Percentage of total vitamin A present in			Ratio Non-liver vit. A Liver vit. A
	Body tissues including head and skin	Viscera	Liver	
		<i>A. aucklandi</i>		
0.37	35	2.5	62.5	0.60
0.50	49	2.7	48	1.09
0.79	59	2.3	39	1.55
1.2	63	1.7	35.5	1.82
1.6	65	1.3	32.5	2.04
2.0	77.5	1.7	21	3.81
3.0	73.5	2.6	24	3.12
3.9*	46.5	0.4	53	0.88*
		<i>A. vulgaris</i>		
0.39	63	2	35	1.9
1.26	69	2.2	28.9	2.4
1.53	60	1.6	38.3	1.6

* See Tables 4*a* and 4*b*.

non-chromogenic or otherwise abnormal substances which may possess vitamin A activity are ignored. Subject to these reservations, however, the potency of the various materials may be considered as of the order 20–25 International Units per g. for each mg./100 g. of “vitamin A” content.

DISCUSSION

In view of previous work on the liver oils of halibut [Lovern *et al.*, 1933], cod, and American plaice (*Hippoglossoides platessoides* Fab.) [MacPherson, 1932–34; 1933] it was expected that the vitamin A content of eel liver oils would also tend to increase with the age and size of the fish. This tendency is in fact exhibited in some degree but it is by no means as pronounced as had been anticipated. It is clear from the data that if the whole of the vitamin were stored in the liver the oils would indeed show a well-marked progressive increase in potency with age, even if the oil content of the liver simultaneously increased considerably, as it does in many species. But in these eels other tissues form a depot for vitamin A in addition to, and apparently at the expense of, the liver. An increase in the oil content of the body tissues seemingly favours this transference from liver to body. The skin, however, despite a high oil content, accumulates little vitamin A, and the head rather less in some cases than would be anticipated from the already lower oil content. There thus appears, in effect, to be some kind of selective mechanism at work, influenced to a varying extent by the oil content. (From the data a general picture emerges of the available vitamin A being “partitioned” between the several organs almost as if between a number of immiscible solvents—although naturally the analogy cannot be pressed very far.) The single extremely lean specimen in Tables IV and VII is an interesting example of the effect of low body oil content. Starvation is well known to result in a scanty but rich liver oil because the oil reserves are utilized more rapidly than the vitamin [Zilva *et al.*, 1924; Drummond & Hilditch, 1930]. In this eel, whatever may be the cause of its leanness, the result has been to transfer a large proportion of the vitamin A from the oil-depleted body back to the liver, so reverting to the conditions which obtain in the smaller eels of this series. It may be significant that the striking tendency shown by *A. aucklandi* for the ratio

$\frac{\text{Non-liver vitamin A}}{\text{Liver vitamin A}}$ to increase with age is also accompanied by a progressively increasing body oil content. No such correlation has yet emerged from our briefer study of *A. vulgaris*. The body tissues of this eel are richer in vitamin A than those of *A. aucklandi* of equal size; this may be because, being smaller by nature, such specimens are more nearly fully grown.

It is not uncommon for the blue value of a weak oil to be doubled or trebled when natural inhibitors are removed by saponification and the colour data calculated back to the original weight of oil. A negative test on the part of an oil has hitherto been regarded as indicating the absence of vitamin A, but in this investigation we encountered two oils which gave no blue colour at all with antimony trichloride before saponification and a pronounced blue colour afterwards. These were the first instances of complete natural inhibition to be observed by us.¹ Since then we have examined three other fish oils giving no colour reaction as oils (Table VIII). Two of these again showed distinct evidence of vitamin A after saponification; the third, since no 620 m μ band could be discerned, probably contained no vitamin A, but it was impossible to confirm this more definitely by the reaction with alcoholic hydrogen chloride [Edisbury *et al.*, 1932] on account of the presence of excessive irrelevant absorption.

Table VIII. *Oils which gave no colour reaction before saponification*

	Spectroscopic colour test via non-sap.		Vitamin A content of oil mg./100 g.
	$E_{1\text{cm.}}^{1\%} 620 \text{ m}\mu$	$E_{1\text{cm.}}^{1\%} 583 \text{ m}\mu$	
Eel body oil*	0.196	0.105	3.9, 4.0
Eel visceral oil*	0.71	0.41	14, 16
Herring (entire) oil	0.095	0.063	1.9, 2.5
Halibut body oil	0.013	0.014	> 0.26, 0.55
Salmon body oil	Absent	> 0.01 ill-defined	Negligible in this specimen

* From small specimens of *A. aucklandi*.

It will be seen that, at very low concentrations of vitamin A, inhibition sometimes assumes significant proportions even in unsaponifiable extracts (cf. halibut body oil) and it is conceivable, though unlikely, that this is the reason for our inability to detect a 620 m μ band in the salmon sample. Removal of sterols by freezing in methyl alcohol usually reduces residual inhibition but is apt in small scale experiments to result in serious loss of vitamin. The last three oils in Table VIII were from individual fish and may not therefore be representative, but it is worth noting that the figures indicate a vitamin A content of 0.08 mg./100 g. for the flesh of this particular halibut, and 0.3–0.5 mg./100 g. for the whole herring. It is perhaps also of interest to mention here that the body oil of the river lamprey *Petromyzon fluviatilis* gave a weak blue colour with antimony trichloride:

Lamprey body oil itself	$E_{1\text{cm.}}^{1\%} 612 \text{ m}\mu$, 0.18;	575 m μ , 0.17	—
Via non-sap.	$E_{1\text{cm.}}^{1\%} 620 \text{ m}\mu$, 0.40;	583 m μ , 0.23;	328 m μ , 0.135
Calc. vitamin A content of oil mg./100 g.	8.0	8.4	8.4

¹ Mittelman [1925; 1927; summarized by Bezssonoff, 1929] reported that the oil from newly autoclaved fresh cod livers gave no colour reaction, but that it acquired normal chromogenic properties on ageing or mild oxidation. Repeating this work, Lovern *et al.* [1931] confirmed that the ability to give a blue colour increases as Mittelman had shown, but they failed to obtain any completely non-chromogenic oil.

corresponding with about 0.7 mg./100 g. of vitamin A for the entire body; and that in the light of present knowledge we should estimate the vitamin A content of the non-liver oils referred to at the beginning of this paper to be:

Conger eel peritoneal fat, <i>ca.</i>	5–10 mg./100 g.
Sturgeon peritoneal fat, <i>ca.</i>	15 ,,
Sturgeon pancreatic fat, <i>ca.</i>	15 ,,

The following figures may be quoted as typical for purposes of comparison:

Halibut liver oil	170–10,000 mg./100 g. (average <i>ca.</i> 2000)
Cod liver oil	20–100 ,, (average 40–60)
Haddock and codling liver oil	4 mg./100 g.

while the activity of good summer butter, expressed as if due solely to vitamin A, corresponds with about 2 mg./100 g. on this basis.

The vitamin D aspect of the problem is not (for the present, at least) amenable to spectroscopic study, but some figures from recent literature seem when taken in conjunction with our own results to be highly suggestive. Ward [1936] quotes 1400 International Units of vitamin D per g. as typical for liver oil from *Anguilla* sp., while Cunningham [1935] reports 47 I.U. per g. for whole specimens of *A. australis*, a species of eel extremely closely related to *A. aucklandi*. The livers of *A. aucklandi* examined by us represented on an average 1.2% of the weight of the entire eel, and contained 1.2–8.1% of oil; corresponding limits for *A. vulgaris* were 1.25–3.2% and 2.05–20.5% respectively. Similar data were not published for Cunningham's eels, but it seems improbable that they lie outside both these sets of limits and most likely that they resemble closely the *A. aucklandi* figures. Accepting this, it follows that either (i) the liver oils were exceptionally rich in vitamin D (7000–200,000 I.U. per g. on the *A. vulgaris* basis, or 50,000–300,000 I.U. per g. on the more probable *A. aucklandi* basis); or (ii) the non-liver tissues form a considerable depot for vitamin D in addition to vitamin A. The subject appears to merit further investigation.

SUMMARY AND CONCLUSIONS

1. In the eels *Anguilla vulgaris* and *A. aucklandi* substantial quantities of vitamin A occur not only in the liver but also in other tissues, and evidence is adduced from recent literature that the same may be true for vitamin D.

2. The distribution of vitamin A is approximately: liver tissues 20–60% of the total; body 25–65%; skin *ca.* 10%; viscera 2%; head 1½%.

3. The concentration in the body tissues tends to increase with age, and in *A. aucklandi* is correlated with and possibly dependent on the amount of oil present.

4. The concentration in the liver oil and in whole liver fluctuates but shows the same tendency to increase with age, though to a less degree. In *A. aucklandi* the potency is often of the same order as halibut and usually greater than in *A. vulgaris*. The liver oil is scanty.

5. The ratio $\frac{\text{Non-liver vitamin A}}{\text{Liver vitamin A}}$ is variable (limits so far encountered 0.6 and 3.8, usually >1.0) and in *A. aucklandi* tends to increase with age.

6. Oils approaching the vitamin A content of cod liver oil are not uncommonly obtained from eel non-liver tissues, while the body (edible) tissues themselves compare favourably with good summer butter.

7. Non-liver oils from other fish—including conger eel, sturgeon, halibut, herring, lamprey—also contain appreciable quantities of vitamin A.

8. For very weak materials substantially free from carotenoids the antimony trichloride colour test is frequently more suitable than the ultraviolet absorption method for the quantitative estimation of vitamin A. Complete inhibition of the blue colour is occasionally encountered, a fact which emphasises the need for saponification in the routine examination of weak oils.

One of us (J. R. E.) is indebted to the Medical Research Council for a full time personal grant.

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