XXI. THE DISTRIBUTION OF VITAMIN A AND FACTOR A_2. I

By J. R. EDISBURY, R. A. MORTON, G. W. SIMPKINS and J. A. LOVERN

From the Chemistry Department, University of Liverpool and the Torry Research Station, Aberdeen

(*Received 30 November 1937*)

The present paper is the full account of work briefly reported elsewhere [Lovern *et al.* 1937; Edisbury *et al.* 1937]. The main experimental result is that fish viscera, and notably the pyloric caeca and intestines, are often very rich in vitamin A. Large deposits of vitamin in the absorptive parts of the alimentary tract make it necessary to study the possibility that vitamin A plays an essential part in food assimilation in some species. This idea is discussed in a preliminary fashion, whilst other aspects of the subject such as the physical properties of visceral oils, vitamin D content, phospholipid content, glyceride composition etc., await further study.

Euler *et al.* [1928; 1929] showed that carotene displays vitamin A activity, in accordance with an earlier suggestion made by Steenbock. The accepted chemical structures and the known physical properties of β-carotene and vitamin A are consistent with a process

$$\underset{\beta\text{-carotene}}{C_{40}H_{56}} \xrightarrow{2H_2O} \underset{\text{2 vitamin A}}{2C_{20}H_{29}OH}$$

first recognized *in vivo* by Moore [1929, 1, 2, 3]. His proof that the administration of a diet rich in carotene results in the rat in a greatly increased accumulation of vitamin A in the liver has been confirmed and extended to fowls and rabbits [Drummond *et al.* 1930; Capper, 1930, 1, 2; Capper *et al.* 1931; Wolff *et al.* 1930].

Biological assays [Morgan *et al.* 1935; Pritchard *et al.* 1937; *M.R.C. Sp. Rep.* No. 202, 1935] carried out on rats suffering from vitamin A deprivation, show that β-carotene and vitamin A are weight for weight about equally effective in restoring growth. This suggests a quantitative conversion of β-carotene into vitamin A when the smallest doses of carotene are given which will produce a satisfactory response.

The important work of Wald [1934; 1935, 1, 2, 3; 1936, 1, 2; 1937, 1, 2] on the vitamin A cycle in low intensity vision reveals a great deal concerning the intimate mechanism of one process in which the vitamin is utilized.

The detection and estimation of carotene and vitamin A in large or small quantities are now on a fairly satisfactory basis [cf. Morton, 1935; 1937] when direct absorption spectra data and/or spectroscopic evaluation of the antimony trichloride colour test are possible, and the application of strict spectroscopic criteria to the promising synthetic materials obtained by Heilbron and others [Davies *et al.* 1935; Heilbron *et al.* 1936; Batty *et al.* 1937; Kuhn & Morris, 1937], will be awaited with interest. Although gratifying progress has been made, the general situation is still unsatisfactory in many ways:

I. Inadequate supplies of vitamin A are demonstrably a factor in widespread malnutrition, so that the value of the vitamin in preventive medicine is manifest.

On the other hand, the conditions for its effective use as a therapeutic agent are less clearly established. Vitamin A is often administered when there is no reason to suspect depletion of the normal liver reserves, and recent advances in the direction of a sharp criterion of suboptimal resources [cf. Jeans & Zentmire, 1934] are very welcome. There is some evidence of immobilization of liver reserves [Mellanby, 1934] and of beneficial effects (e.g. in disseminated sclerosis) not clearly connected with the relief of frank deficiency.

II. The known precursors of vitamin A all possess the β-ionone ring and the characteristic side chain, but it would be premature to assume either that no new provitamins A remain to be discovered or that the structure must be in large measure preformed before the *in vivo* transformation can occur. Neither the mechanism nor the site of synthesis is established. The claims to have achieved the conversion of carotene into vitamin A *in vitro* under the action of a liver carotenase are not in our experience verifiable [cf. Rea & Drummond, 1932; Woolf & Moore, 1932].

The work of Drummond and his colleagues on mammals [Drummond & Macwalter, 1933; 1934; Drummond *et al.* 1934] seems to eliminate the whole of the alimentary tract from the possible sites of synthesis *in vivo*. When carotene was introduced into the circulation, it was rapidly taken up by the liver and disappeared in a few days. Experiments involving partial hepatectomy followed by introduction of carotene showed that operation-shock of itself brought about a large fall in the vitamin A reserves of the remaining liver lobes. Suggestive as this phenomenon is from the point of view of vitamin A therapy, it prevents an acutely planned experiment from achieving its immediate object of settling whether the liver is or is not the site of vitamin A synthesis. The prevailing idea that provitamins A (carotene, etc.) ingested orally are absorbed from the intestine and conveyed to the liver for (possibly enzymic) hydrolysis to vitamin A, is at present no more than an attractive hypothesis. The objections to it are perhaps most serious for the general case of fishes (*vide infra*) where the vitamin A concentration attains levels difficult to reconcile with the intake of dietary vitamin A or known provitamins.

III. Another difficulty lies in the disparities in vitamin A content which emerge when different animals and fishes are compared. These disparities are very large, e.g.

	Parts per million vitamin A
Human liver	20
Rabbit liver	70
Halibut liver	2000

Differences of this kind recur within genera and species, although mammals seem to possess lower reserves than many fishes.

It is possible that some species accumulate vast reserves of vitamin in the same way as an experimental rat which is receiving carotene in quantities unnaturally large for a herbivorous animal. Such an idea would perhaps mean that the enormous deposits of vitamin A in, let us say, halibut, are in a sense accidental and without function, harmless possibly, but as irrelevant to nutrition as an intestinal calculus. It should be noted, however, that the diet of the halibut (mixed species of fish) is not appreciably different from that of other fish which do not contain such large quantities of vitamin A.

Alternatively it is necessary to examine the notion that the vitamin can fulfil a variety of functions. The diet of the carnivorous fish consists mainly of fat and protein, and the occurrence of vitamin A in the absorptive sections of the alimentary tract is enough to warrant investigation of the role it plays in

assimilation of either fat or protein or both. Its distribution runs suggestively parallel with the distribution claimed for fat absorption, whilst there is much evidence that at other sites it promotes the proper functioning of epithelial cells "working in harmony with proteins of high biological value" [Mellanby, 1934].

The conception of a large physiological response to a small quantum of accessory food factor is part of the classical notion of a vitamin; there is however no reason to exclude other functions, especially in a fish like halibut which contains many grams of a substance hitherto mentally associated with a minute fraction of a milligramme capable of relieving a condition of outspoken deficiency. When, as is so often said, the animal is to be regarded as the final court of appeal in vitamin research, it must be remembered that the statement has so far implied the prevention or cure of avitaminosis through the response to minimal doses. Unless we are to adopt the hypothesis of fortuitous accumulation, there are obvious attractions in the idea of grosser processes requiring much larger amounts than those needed to prevent or counteract recognized deficiencies. It will be useful at this stage to draw a theoretical distinction between (*a*) "classical" conceptions of the functions of vitamins, and (*b*) newer conceptions of functions involving relatively large absolute quantities of vitamin.

Clinical tests with concentrates might well be reviewed in the light of such a distinction.

IV. The quantitative conversion of carotene (or other provitamin) into vitamin A may not occur without hitherto undetected intermediate stages, a possibility which complicates the problem of recognizing the site or sites of the processes.

V. More immediate from the present point of view are the difficulties concerning the antimony trichloride colour test. The blue solution obtained with vitamin A shows $\lambda_{max.}$ 620mμ., displaced to 605mμ. in weak oils. Since 1929 repeated observations of a maximum near 693mμ. have been recorded [Morton *et al.* 1931; Heilbron *et al.* 1931; 1932; Edisbury *et al.* 1932; Lederer & Rosanova, 1937; Edisbury *et al.* 1937; Heilbron *et al.* 1937] and sharp bands at 635–640 and 660mμ. have occasionally been seen. These chromogens may be akin to vitamin A. They have given rise to speculation as to their structure, and misgivings have been felt that accepted ideas about the physiological roles of carotenoids and polyene alcohols are much too simple. The work of Wald [1937, 1, 2] on porphyropsin anticipates our own work [Edisbury *et al.* 1937]; together they suggest that the 693mμ. chromogen may become recognized as a new vitamin. In the present paper this chromogen is referred to as factor A_2. There is no evidence that its function as a vitamin extends beyond fishes.

VI. Lastly, the importance of the liver as the main depot for vitamin A is now greatly reduced. Some rather neglected observations by Rosenheim & Webster [1927] on eels suggested as much, whilst later work [Edisbury *et al.* 1937] confirmed a wide dissemination of the vitamin over the eel body as a whole. Non-liver tissues of other species have also been found to contain substantial quantities of vitamin A, often exceeding the total liver reserves (*vide infra*) [cf. Shorland, 1937].

The following notes are intended to assist the perusal of the experimental sections.

For purposes of calculation the criteria:

$E^{1\%}_{1\,cm.}$		
620mμ.	5000	Colour test
583	2600	
325	1600	Ultraviolet absorption

[Carr & Jewell, 1933; Morton, 1935]

may be taken to represent the intensities of the absorption maxima characteristic of practically "pure" vitamin A. Thus an oil showing

$E^{1\%}_{1\,cm.}$	
620 mμ.	50
583	26
325	16

contains 1% of vitamin calculated as free alcohol (or correspondingly more if calculated as ester). Some slight revision may be necessary if various claims to have prepared rather purer materials are substantiated. For the present the above figures afford a convenient basis for comparison.

Factor A_2 has not been isolated in a state approaching purity, so that strictly quantitative criteria are lacking. Approximate criteria can be obtained from data on sterol-free concentrates containing over 75% of vitamin A:

$E^{1\%}_{1\,cm.}$ 693 mμ.	$E^{1\%}_{1\,cm.}$ 620 mμ.	% vitamin A	% factor A_2 by difference
671	4325	86·5	> 13·5
594	4250	85	> 15
720	4150	83	> 17
945	3813	76·2	> 23·8
1050	3820	76·4	> 23·6
1000	4000	80	> 20

Although it is not safe to assume that factor A_2 is the sole contaminant of these very rich vitamin A concentrates, substantial amounts of other impurities are very unlikely. The figures indicate that for 100% factor A_2 $E^{1\%}_{1\,cm.}$ 693 mμ. will be of the order of 5000.

Provisionally, therefore, $E^{1\%}_{1\,cm.}$ 5000 in the colour test can be accepted for both vitamin A and factor A_2 although it is recognized that the results may ultimately have to be recalculated.

Saponification technique. The simplest and probably most accurate evaluation of the total vitamin A in tissues is obtained by direct saponification of the whole material, or of a representative sample, by means of alcoholic KOH.

To every 3 g. of tissue add 1 ml. 60% aqueous KOH and 10–20 ml. absolute alcohol, and heat on a water bath. Loss of solvent may be avoided by adding more alcohol, but this is rarely necessary. The amount of alkali represents considerable excess; this is necessary to ensure complete disintegration of tissue and saponification of fat in 1–2 hr. Preliminary extraction of tissue in order to determine the oil content is best performed as a separate experiment, especially if the vitamin content is low. With small amounts of material it is not usually worth while to determine oil, and vitamin estimations carried out on oils rather than on direct non-saponifiable extracts may err on the low side. With oils of low potency, preliminary preparation of "non-sap." is essential, and 0·5 ml. 60% KOH and 10 ml. alcohol are used for each g. of oil. Saponification at 100° is continued for 5–15 min. The hot solution of saponification products (whether from tissue or oil) is diluted with twice its volume of cold water and extracted twice with 3 times its volume of freshly redistilled (peroxide-free) ether. Further extraction is only occasionally necessary. The combined extracts are washed as follows: water, dilute alkali, water, dilute alkali, water 3–4 times [cf. S.P.A. technique, *Analyst*, 1933, **58**, 203; Morgan *et al.* 1935; *M.R.C. Sp. Rep.* No. 202, p. 52]. Aliquot portions are taken for the colour test and for the measurement of ultraviolet absorption. The ether is distilled off and the slightly wet residue is made up to known volume for ultraviolet absorption with absolute alcohol. For the colour test, moisture is first removed by twice adding a few drops of alcohol and "blowing" with nitrogen or carbon dioxide from a cylinder, while the flask is warmed on a water bath. The dry residue is then dissolved in a known volume of pure chloroform. Small quantities (0·2–0·4 ml.) are treated with the antimony trichloride reagent and the blue colour is studied spectroscopically.

Vitamin A and factor A_2 in eyes

The determinations were made on light-adapted eyes, and retinae were not examined separately, since the object of the work was to ascertain the absolute amount of vitamin present in whole eyes. It was hoped that the information

gained would help to distinguish organs or sites of storage, and sites of utilization, in a process involving true vitamins.

It is unnecessary (and in fact undesirable) to isolate the fat before determining vitamin A in eyes. The whole eyes are treated with alcoholic KOH until disintegration is complete (1–2 hr.). The lenses separate cleanly at an early stage and are only slowly attacked by alkali. The choroid is even more resistant. The non-saponifiable fraction is extracted in the usual way (*vide* p. 121) and although the amount is barely visible, satisfactory tests are possible.

The extracts obtained did not all give unequivocal evidence of the presence of vitamin A; thus a batch of brown trout (*Salmo trutta* v. *fario*) eyes and the eyes from one specimen of sea bream (*Pagellus centrodontus*) yielded pale yellow extracts giving a slate blue colour with the antimony trichloride reagent. The blue solutions showed a faint absorption maximum at 590–595mμ. which indicated the presence of a little carotenoid, but scarcely any vitamin A. All the other non-saponifiable extracts from eyes gave, however, positive tests for vitamin A, the concentrations (expressed in parts per million of whole eye tissues) remaining remarkably constant over a number of species.

The eyes of goldfish (*Carassius auratus*) proved exceptional and yielded 10–20 times the "normal" quantity of vitamin A. The concentrations observed were in fact appreciably higher than in some liver tissues (e.g. a human liver). One specimen contained nearly as much vitamin A in the eyes as in the rest of its body. The rich extracts from goldfish eyes exhibit a well-defined ultraviolet absorption spectrum. This is naturally affected by extraneous absorption due to substances other than the main absorbing entity. The colour test also exhibits a well-defined absorption spectrum influenced in some degree by substances other than the principal chromogens, and there is evidence that one chromogen can interfere with another and cause partial inhibition. Extraneous absorption in the ultraviolet raises the intensity of absorption and inhibition in the colour test depresses the readings. In consequence of these two tendencies, a rather wide margin of uncertainty sometimes appears in the estimates of vitamin content (see Table I).

Goldfish eyes differed materially from those of sea fish in that the maximum in the antimony trichloride colour test occurred at 690–695mμ. rather than 610–620mμ. (green colour instead of blue). The non-saponifiable extracts from goldfish eyes, dissolved in absolute alcohol, exhibited ultraviolet absorption spectra with maxima at *ca.* 350 and 288mμ. as distinct from the single maximum at 325mμ. associated with vitamin A. It is well known [Edisbury *et al.* 1932; Pritchard *et al.* 1937] that under the action of alcoholic HCl the vitamin A molecule undergoes cyclization whereby the broad unresolved band at 325mμ. is replaced by a group of narrow bands:

$$\lambda_{max.}\ 392,\ 369,\ 350 \text{ and } 333\text{m}\mu.$$

Cyclization of the extract from goldfish eyes gave

$$\lambda_{max.}\ 391,\ 369,\ 349 \text{ and } 334\text{m}\mu.$$

The apparent identity of the cyclization products makes it clear that the structural difference between the 620 and the 693mμ. parent chromogens can only be small.

Wald [1937, 1, 2] has shown that the 693mμ. chromogen can replace vitamin A in the visual purple cycle. He refers to the pigment in the eyes of freshwater fishes as porphyropsin to distinguish it from rhodopsin. The 693mμ. chromogen also occurs in the livers and viscera of a great variety of sea fish, but is accom-

panied by a considerably greater quantity of vitamin A. In a number of freshwater fishes the proportions are almost reversed, particularly in the liver [cf. Lederer & Rosanova, 1937; Heilbron *et al.* 1937].

Vitamin A is sometimes replaced by the 693 mμ. chromogen, referred to as factor A_2, in fishes' eyes, liver and viscera (pyloric caeca, intestines, spleen etc.). The chromogen does not seem to occur in mammals and feeding tests on mammals will be difficult to interpret. A negative response to avitaminosis-A would be expected unless conversion into vitamin A occurred *in vivo*. In view of the structural similarity deduced for vitamin A and factor A_2 such a change is not impossible. Biological experiments on freshwater fishes are needed to ascertain the true role of factor A_2 apart from its function in vision.

Table I. *Vitamin A content of eyes*

	Number of eyes	mg. vitamin A per eye	Vitamin A concentration: parts per million of whole eye
Rabbit (*Lepus cuniculus*)	6	0·008	3
Haddock (*Gadus aeglefinus*)	6	0·003–0·0033	3·9–4·3
Whiting (*G. merlangus*)	10	0·01–0·014	4·5–6·1
Hake (*Merluccius vulgaris*)	12	0·022–0·023	2·6–2·7
Halibut (*Hippoglossus hippoglossus*)	4	0·0037	5·5
Herring (*Clupea harengus*)	36 (small)	0·0011	1·5
,,	21 (adult)	0·004–0·005	3·3–4
,,	12* (adult)	*ca.* 0·008	*ca.* 7
Goldfish (*Carassius auratus*), large variety	2	0·001–0·002	5–10
Goldfish (*C. auratus*), small variety	2	*ca.* 0·002	*ca.* 60
,, ,,	6	0·0016	50
,, ,,	10	0·0012	*ca.* 40
,, ,,	8	0·0019–0·0053	36–100
,, ,,	24	0·003	90

* Oil content 6·4%.

Distribution of vitamin A in the rabbit (Lepus cuniculus)

In this study it was hoped to ascertain the distribution of vitamin A in a mammal living on a natural diet. A herbivorous mammal was preferred to a carnivore since the latter would receive dietary vitamin A, whilst the rabbit receives only provitamin A (carotene) and that in relative abundance.

Three freshly shot rabbits were brought to the laboratory without delay and dissected. The different parts were combined and weighed (3 livers, 3 spleens etc.) and at once covered with alcoholic KOH. The non-saponifiable extracts were obtained and tested for vitamin A.

From experiments on rats, it is clear that when the need for vitamin A is acute, the mammalian organism achieves an almost quantitative conversion of β-carotene into vitamin A. On a carotene-rich diet the rat synthesizes and retains relatively large quantities of vitamin, whereas rabbits shot at midsummer and as replete with carotene as they would ever be in a state of nature, retain relatively little. This is in striking contrast with the data on halibut and other fishes.

The requirements of the rabbit in respect of vitamin A are very modest. It will be seen from Table II that about 95% of the total vitamin is present in the liver. In the female, the mammary gland is the next most important depot, or rather entrepot, since the store covers no more than a day or two's

Table II. *Vitamin A in the rabbit*

Tissues*	Wt. g.	$SbCl_3$ test	Vitamin A mg.	Concentration in tissue parts per million
Eyes	14·4	Clear max. 614 mμ.	0·046	*ca.* 3
Stomachs	58	Faint max. 585 mμ.	0·002 ?	—
Spleens	3·3	Negative	None	—
Hearts and lungs	53·3	Faint blue	0·005 ?	—
Mammary gland	72	Clear 614 mμ. max.	0·094	*ca.* 1
Pancreas	6·5	Faint max. 602 mμ.	0·0045	—
Mesentery	12·7	585 mμ. max.	0·01 ?	—
Gonads, Uteri, Bladders	13·3	Faint slaty blue	Trace only	—
Small intestines	150	Maxima at 617 and 585 mμ. clear	0·01–0·02	*ca.* 0·1
Large intestines	140	Maxima at 617 and 585 mμ. clear	0·3	2
Kidneys and suprarenals	34	Clear max. 615 mμ.	0·1	3
Livers	160	Good 617 mμ. band	11·0	*ca.* 70

* From 3 rabbits, except that only one mammary gland was used.

Liver vitamin A/Total vitamin A = 95%. 693 mμ. chromogen not observed.

supply for lactation. It may be significant that the large intestine contains approximately 2 p.p.m., suggesting a "classical" function akin to that obtaining in the eyes, which have 3 p.p.m.

Distribution of vitamin A in the herring (Clupea harengus)

The main interest of this work lies in the occurrence of relatively large quantities of vitamin A in the alimentary tract. In the herring the pyloric caeca are numerous and form a substantial, fairly compact mass.

The pyloric caeca are caecal appendages or outgrowths from the intestine, situated close to the pyloric extremity of the stomach and the intestinal apertures of the bile and pancreatic ducts. Absent from some species of fish, they are subject to extraordinary variations in number, size and arrangement. Many functions have been ascribed to the pyloric caeca; fat absorption seems to be one of the most important.

These appendages contain vitamin A in quantities comparable with those found in the liver, and if the portion of the intestines comprising the caeca is boiled with water a pleasant smelling oil is easily obtained and is found to be similar to cod liver oil in vitamin A potency.

The herring, as is well known, varies enormously in oil content; the sex cycle, the season and the diet being concerned in varying degree.

The figures given below may not be representative of the main catches. They make it clear however that more tests are needed on fish caught at different seasons and in different waters. The annual herring landings from British vessels (*ca.* 8,000,000 cwt.) represent more than twice the weight of the next largest catch (cod).

So far as the tests have gone, the viscera usually contain more vitamin A than the liver, although this is not always the case (see Exp. 4). The visceral oils are easily obtained but the potency is relatively low on account of the abundance of oil itself.

The 693 mμ. chromogen, factor A_2, is clearly present in herring oils, but the intensity of the band in the colour test is only *ca.* 1/6 that of the 620 mμ. band of vitamin A. A feeble colour test $\lambda_{max.}$ 590 mμ. is shown by material obtained

from stomachs, but is due either to minute traces of vitamin A in the presence of much natural inhibitor [cf. Lovern *et al.* 1931] or, more probably, to traces of carotenoids.

Table III. *Distribution of vitamin A in herrings*

Exp.	Material		Wt. g.	Wt. vitamin A mg.	Concentration of vitamin A parts per million	Wt. oil g.
1	1 entire fish		124	*ca.* 0·4	3·0	13
2	6 herrings	(Mean)	256	—	—	—
	Gonads	(Mean)	44·3	Negligible	—	—
	Eyes	(Each)	1·15	Trace	—	—
	Viscera, including liver and gills	(Mean)	13·5	0·12	9·0	0·40
	Body		—	None	—	—
3	1 herring ♀		283	—	—	—
	Body		205	None	—	—
	Liver		2·45	0·037	15	—
	Gills		5·1	Trace	—	—
	Gonads		64·1	Not tested	—	—
	Rest of viscera		6·7	0·08	12	—
4	1 herring ♀		—	—	—	—
	Body		172	None	—	—
	Liver		1·7	0·5	293	—
	Gonads		43	—	—	—
	Viscera		6·7	0·32	48	—
	Gall bladder		0·1	0·003	30	—
5	6 herrings		980	—	—	—
	Total viscera		101	2·6	25·8	45·4
6	20 herrings		—	—	—	—
	Pyloric caeca		42	3·36	80	—
	Livers and gall bladders		40	2·2	55	—
	Oesophagi and stomachs		36	(590 mμ.) Trace	—	—
	Intestines		20	0·4	20	—
7	20 herrings		—	—	—	—
	Pyloric caeca		47	7·2	153	5·5

Table IV. *Oils from herrings*

Exp.	Material	$SbCl_3$ test	Approximate vitamin A potency in I.U./g.
2	Visceral oil	Normal; carried out on "non-sap" $\lambda_{max.}$ 620 mμ.	300
5	Visceral oil	Normal; tests on non-sap. calc. on oil $E^{1\%}_{1\,cm.}$ 693 mμ. 0·04; 620 0·29; 583 0·16	150
7	Oil from pyloric caeca	Tests on oil show inhibition like cod liver oil; tests on oil $E^{1\%}_{1\,cm.}$ 693 mμ. 0·8; 612 4·2; 580 3·1	2150

The preliminary work was carried out on material purchased from Liverpool retailers, and the later work was done on materials kindly furnished by the Staff of the Marine Biological Station (University of Liverpool) Port Erin, Isle of Man.

Distribution of vitamin A in salmon (Salmo Salar)

A fresh salmon (River Dee, Wales) was eviscerated and the "pluck" divided into three portions for vitamin A assay. Similar tests were made on the viscera of frozen Canadian salmon.

Table V

	One fresh salmon			Three frozen salmon		
	Wt. g.	Vitamin A mg.	Concentration p.p.m.	Wt. g.	Vitamin A mg.	Concentration p.p.m.
Pyloric caeca	25·9	35	1353	718	200	278
Liver	59·4	10	168	224	100	446
Rest of viscera	38	8·7	229	Intestines stomach	30 Trace	—

	Fresh salmon			Frozen salmon		
$E^{1\%}_{1\,cm.}$ *	693 mμ.	620 mμ.	583 mμ.	693 mμ.	620 mμ.	583 mμ.
Pyloric caeca	0·9	6·8	3·5	0·135	1·4	0·72
Liver	0·93	0·93	—	1·76	2·17	1·84
Intestines	0·16	1·16	0·56	0·2	2·1	1·1
Stomach	—	—	—	0·0012	0·0082	—

* Calculated on the wet weights immediately after dissection.

In the salmon family the pyloric caeca are very numerous and large in relation to the rest of the alimentary tract. They contain more vitamin A than the liver and the rest of the viscera combined. The stomachs contain only traces of vitamin, whereas the intestines have moderately large amounts.

The 693 mμ. chromogen occurs both in the liver and the alimentary tract, but whereas the ratio $E^{1\%}_{1\,cm.}$ 693/$E^{1\%}_{1\,cm.}$ 620 mμ. is approximately 1 for the liver, it falls to approximately 0·1 in the other viscera. Salmon livers are thus a good source of factor A_2, but the viscera yield oils corresponding closely with the more familiar fish liver oils. The 693 mμ. chromogen tends to preponderate over vitamin A in the livers of freshwater fishes [cf. Lederer & Rosanova, 1937; Heilbron *et al.* 1937]. In this respect salmon resembles the true freshwater fishes. The distribution of factor A_2 is shown below:

	1 fresh salmon	3 frozen salmon
Pyloric caeca	5	20
Liver	10	70
Rest of viscera	1·2	3

If $E^{1\%}_{1\,cm.}$ 693 mμ. = 5000 for factor A_2 the above figures represent mg. (but see p. 121).

The results on frozen salmon can be stated in another way.

Livers (224 g.) ≃ 1·5 g. non-sap. (partially sterol free) pyloric caeca (718 g.) ≃ 12 g. oil separated, residue yielded 1·34 g. non-sap.

		Liver "non-sap."	Pyloric caeca "non-sap."
Colour test	$E^{1\%}_{1\,cm.}$ 693 mμ.	236	64·2
		615 325	660
		580 163	360
Ultraviolet absorption	$E^{1\%}_{1\,cm.}$ 353 mμ.	50	
		327 89	
		288 50	

i.e. liver "non-sap." contains about 6% vitamin A and <6% factor A_2, while pyloric caeca "non-sap." contains about 13% vitamin A and <1·3% factor A_2.

Vitamin A and factor A_2 in trout (Salmo trutta *v.* fario; S. irideus)

Six brown trout (*S. fario*) were eviscerated soon after being caught in an Irish river and the "pluck" was at once placed under alcohol. The livers were later separated from the rest of the viscera, and the combined portions were weighed, saponified completely and the non-saponifiable matter extracted.

	Livers 8·3 g.			Other viscera 53 g.	
$*E^{1\%}_{1\,cm.}$	696 mμ.	0·157	Colour test	693 mμ.	0·04
	620	0·10	,,	620	0·027
	351 mμ.	0·08	Ultraviolet absorption	346 mμ.	0·023
	288	0·084	,, ,,	288	0·025

* Calculated on the weight of tissue.

The 693 mμ. chromogen was more powerful than the 620 mμ. chromogen in both fractions, and the ultraviolet absorption spectra showed two bands with maxima at 346–351 mμ. and 288 mμ. instead of the normal vitamin A maximum at 325 mμ.

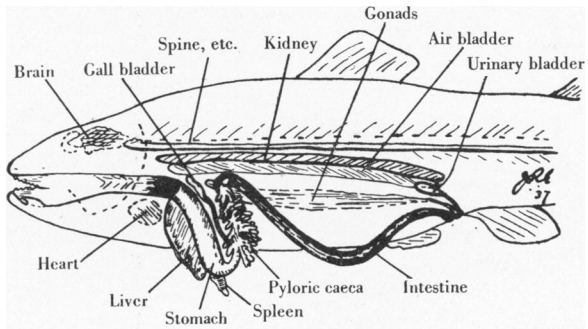

Fig. 1. A typical Teleostean (trout), showing pyloric caeca in relation to other organs.

A single brown trout was then dissected and various organs were worked up separately. The 693 mμ. chromogen again predominated, and it is estimated that the whole fish contained 0·25 mg. vitamin A and 0·5 mg. factor A_2.

	Liver		Pyloric caeca	Gonads
$E^{1\%}_{1\,cm.}$	690 mμ.	0·068	0·085	A small quantity only
	620	0·05	0·039	of vitamin A. 693 mμ.
	346	—	0·034	band not seen

The apparent correlation between the 346 mμ. maximum in direct absorption and the 693 mμ. chromogen is maintained.

A specimen of sea trout (*S. trutta*) was next examined.

	Liver		Pyloric caeca	Intestines
$E^{1\%}_{1\,cm.}$	690 mμ.	0·82	0·7	A trace only
	620	0·82	2·25	of vitamin A
	580	0·42	1·15	

The sea trout resembles the salmon rather than the river trout in respect of the amount and distribution of both chromogens.

A larger supply of material was obtained from the Liverpool Corporation Reservoir at Vyrnwy. By the courtesy of Captain Martin of the Lake Vyrnwy Hotel we were enabled to dissect the viscera of 8 rainbow trout (*S. irideus*) and 7 brown trout, freshly caught and placed in a refrigerator. The different portions of the viscera were immersed in alcohol and sent to the laboratory.

The pyloric caeca (68 g.) from 7 rainbow trout were treated with a small amount of alcoholic KOH at 100° for a period just long enough to disintegrate the tissues. On cooling, a pleasant smelling oil separated, part of which was decanted off and part extracted by means of redistilled ether. After removing solvent, the oil was centrifuged to remove tissue debris, and tested:

$E^{1\%}_{1\,cm.}$	693 mμ.	2·35
	620	9·4
	580	5·35

equivalent to 310 p.p.m. vitamin A in the wet tissue.

The oily product was thus several times richer in vitamin A (*ca.* 6000 I.U./g.) than cod liver oil, but the potency may have been raised by partial saponification of fat and accumulation of "non-sap" in the residual oil.

In order to ascertain how readily the 693 mμ. chromogen could be concentrated, the 15 livers were worked up as one batch:

161 g. liver ≃ 0·35 g. "non-sap." (sterol mixed with a red oil).

		"Non-sap."	
$E^{1\%}_{1\,cm.}$	690 mμ.	181	*ca.* 4·0% factor A_2
	620	225	*ca.* 4·5% vitamin A
	580	117	

The non-saponifiable matter from intestines (112 g.) gave

$*E^{1\%}_{1\,cm.}$	693 mμ.	0·12
	620	0·44
	580	0·23

* Calculated on tissue weight.

vitamin A preponderating markedly over factor A_2.

The stomachs (148 g.) gave on the same basis:

$E^{1\%}_{1\,cm.}$	693 mμ.	0·0016
	620	0·0045*

* i.e. just measurable quantities of both chromogens.

Table VI. *Distribution in Vyrnwy trout*

	Vitamin A		Factor A_2	
	mg.	p.p.m.	mg.*	$E^{1\%}_{1\,cm.}$ 693/620 mμ.
Stomachs (15)	⊅ 0·15	1	*ca.* 0·05	1 : 3
Pyloric caeca (7 *S. irideus*)	21	310	5	1 : 4
Intestines (15)	10	90	2·5	1 : 4
Livers (15)	16	99	12	1 : 1·25

* See p. 121.

Making an allowance for the vitamin A contained in the 1 rainbow trout and the 7 smaller brown trout for which the figures were not obtained, it is clear that more than half the total occurs in the pyloric caeca and intestines.

In fact the liver contains only 1/3 of the total vitamin A, but 3/5 of the total factor A_2.

Although the concentrations in brown trout are lower than in rainbow trout (the Vyrnwy material was predominantly representative of the latter because the *S. fario* were much smaller than the *S. irideus*), it is still true that 2/3 of the vitamin A occurs outside the liver except in the sea trout.

Table VII. *Distribution of vitamin A in trout*

	One brown trout Vitamin A		Six brown trout from Ireland Vitamin A		Factor A_2	One sea trout Vitamin A		Factor A_2
	mg.	p.p.m.	mg.	p.p.m.	mg.*	mg.	p.p.m.	mg.*
Gonads	0·02	1·5	—	—	—	—	—	—
Pyloric caeca	0·15	17	—	—	—	1·8	138	0·6
Liver	0·7	10	0·166	20	0·4	4·1	164	4·1
Rest of viscera	None detected	0	0·27	5·2	0·26	—	—	—

* See p. 121.

The final experiment in this series on trout was on a rather larger scale, the object being to isolate a moderate quantity of visceral oil under mild chemical treatment.

A fishmonger was provided with a 2 l. brown glass bottle containing alcohol and was asked to fill it with the "pluck" from brown trout. The viscera (including livers) were left to stand for several days in the presence of a little alcoholic KOH. The material was then transferred to a large flask and brought to 100° for a few minutes. Although the tissue was only partially disintegrated, much oil came to the surface. It was extracted by means of redistilled ether, and a good yield of a pleasant smelling orange red oil resulted:

		Oil	
Colour test	$E^{1\%}_{1\,cm.}$	685 mμ.	0·72
		636	0·74
		598	0·63
		557	0·50
Direct absorption spectrum	$E^{1\%}_{1\,cm.}$	475 mμ.	0·12 }
		448	0·15 } Carotenoid?
		421	0·12 }
		353	0·57 Marked inflexion
		325	0·6 „
		282	1·0 Maximum

The oil contains it would seem, vitamin A, factor A_2 and a carotenoid.

The non-saponifiable extract gave

	Oil	
$E^{1\%}_{1\,cm.}$	691 mμ.	1·11
	620	0·61

whilst pigmented acids recovered from the soaps gave a green colour with antimony trichloride, $\lambda_{max.}$ 665, 590, 535 mμ., all transient.

A portion of the "non-sap." was cyclized by the use of alcoholic HCl and the recovered acid-free product was tested with antimony trichloride. Two bands were seen, one at 693 and one at 624 mμ., the latter being clearly defined and distinguishable from the 620 mμ. band of vitamin A. The 624 mμ. chromogen thus appears to be an artefact.

Earlier data on the sturgeon (*Acipenser sturio*) show that the bands at *ca.* 640, 595 and 560 mμ. need not necessarily be accompanied by the 693 mμ. band of factor A_2:

	Liver oil		Pancreatic oil		Peritoneal oil	
$E^{1\%}_{1\,cm.}$	617 mμ.	39	640 mμ.	0·24	640 mμ.	0·12
	583	19	600	0·39	602	0·33
	325	18	565	0·35	568	0·3

Vitamin A in perch (Perca fluviatilis)

A preliminary study has been made of perch from Lake Windermere. All of the fish were fasting prior to death.

Table VIII. (*8 perch*)

	Total wt. g.	Chromogenic values calculated on tissue weights		
Livers	3·27	$E^{1\%}_{1\,cm.}$	693 mμ.	0·04
			640	0·075
			595	Transient
			560	,,
Alimentary tracts	6·98	$E^{1\%}_{1\,cm.}$	693 mμ.	0·008
			640	Transient green colour
Eyes	5·83	$E^{1\%}_{1\,cm.}$	610 mμ.	> 0·003
		Qualitative test for vitamin A		
Other viscera (heart, spleen, gonads, mesentery)	20·3	$E^{1\%}_{1\,cm.}$	695 mμ.	0·010
			636	0·011
			556	0·09

The colour test value for the eyes is unusually low (0·6 p.p.m.) and no factor A_2 was recorded. The fish had been fasting in a rather well lit aquarium and the results may not be entirely normal. The eyes are moreover unusually large in relation to the size of the fish. The bands at 693, 640, 595 and 560 mμ., occurring even in the liver extract show a surprising chromogenic complexity, but the amounts of the chromogens must be very small indeed.

Table IX. *Distribution of vitamin A in turbot* (Psetta (Rhombus) maximus) (*12 fish*)

	Wt. g.	Wt. vitamin A mg.	Concentration vitamin A p.p.m.	% oil	Tests on oil		
Stomach*	450	3·01	6·7	0·79	$E^{1\%}_{1\,cm.}$	610 mμ.	2·2‡
						572	2·2
Duodenum*	135	40·5	300	1·20	$E^{1\%}_{1\,cm.}$	693 mμ.	<13§
						620	125
						583	66
Pyloric caeca†	62	>7‖	>115‖				
Ileum†	53·3	14·4	270				
Mesentery†	23·5	1·42	60				
Rectal caecum†	52·6	7·36	140				
Spleen†	30·9	0·18	6				

* Oils extracted at Aberdeen.

† Tissues placed in alcohol, saponified directly at Liverpool.

‡ The stomach oil is little better than cod liver oil and contains much colour test inhibitor.

§ The duodenal oil contains *ca.* 2·5% vitamin A and thus compares favourably with halibut liver oil. The whole of the intestines would probably give a similar oil.

‖ The turbot has two stumpy pyloric appendages; part of the material was used for other work, so that the above figures err on the low side.

Table X. *Distribution of vitamin A in conger eel* (Conger conger)

The tissues were extracted at Aberdeen, and part of the products sent to Liverpool for assay.

Tissue	Wt. g.	% oil	Wt. oil g.	$E^{1}_{1\,cm.}$ % 620 mμ. on oil	Wt. vitamin A in tissue mg.	Concentration in tissue p.p.m.
Body plus head	8608	11·85	1020	0·154	32	3·72
Liver	116	14·2	16·5	24·0	79	681
Spleen	63	4·1	2·58	> 0·06	0·031	0·5
Stomach	65	0·8	0·52	0·86	0·088	1·35
Intestines	37	1·6	0·59	2·0	0·24	6·5
Mesentery	13	11·7	1·52	0·65	0·20	15·4
Gonads	425	59·2	251·6	0·58	29	68·2

Vitamin A in liver 79 mg.
Vitamin A elsewhere 61·6 mg.
Total 0·14 g.

The above results may be compared with earlier data [Edisbury *et al.* 1937] on the eels *Anguilla vulgaris* and *A. aucklandi*.

Table XI. % *total vitamin A occurring in different parts of the eel*

	Anguilla	*Conger*
Body (including head and skin)	35–75* <	23
Liver	20–60*	56
Viscera	2	0·4
Gonads	(Included in viscera above)	20

* Varies according to age and other factors.

In eels the gonads take the form of strips of adipose tissue, and from data on other fish it is probable that the greater part of the vitamin found in the "gonads" is present in the fat investing the tissue rather than in the gametes themselves.

Table XII. *Vitamin A in spotted ray* (Raia maculata). *River Dee Estuary, Cheshire*

Liver	0·7 p.p.m. vitamin A in tissue
Eyes	Mere trace vitamin A in tissue

The following organs all gave negative results for vitamin A: spleen, pancreas, gonads, stomach, spiral valve and intestines. The extraordinarily low vitamin A content of this large fish requires confirmation.

Table XIII. *Sunfish* (Orthagoriscus mola *or* mola mola)

One fish 3–4 cwt.; oils extracted at Aberdeen. Liver 17·4 kg.; 10·4 kg. oil containing *ca.* 0·75 g vitamin A, or 44 p.p.m. in the liver.

Negative tests for vitamin A were obtained on the following oils:

	% oil		% oil
Flesh	0·08	Stomach*	0·92
Cartilage	0·05	Duodenum*	0·47
Spleen	1·35	Ileum	0·49
Heart	0·95	Caecum	0·51
Mesentery*	0·97		

* The pyloric end of the stomach was filled with worms and much destruction had occurred.

Again, we have a large fish with very little vitamin A.

Table XIV. *Distribution of vitamin A in cod* (Gadus callarias) (*8 fish*). *Aberdeen*

Material	Wt. g.	Wt. oil g.	Vitamin A
Stomach	623	4·55	Nil
Pyloric caeca	331	4·70	26 mg.
Mesentery	28	0·37	Trace
Duodenum	137	1·03	Nil
Ileum	64	0·49	Nil
Caecum	59	0·48	Nil
Spleen*	—	1·3%	56 p.p.m. (tissue)

* Some missing.

	Pyloric caeca oil	Spleen oil
$E^{1\%}_{1\,cm.}$ 693 mμ.	2·0	<5
620	27·6	20
583	15·6	12
	ca. 0·55% vitamin A	*ca.* 0·4% vitamin A

Visceral oils from cod may thus be obtained some 20 times richer in vitamin A than cod liver oil, but the present tests are essentially preliminary.

The distribution of vitamin A in the halibut (Hippoglossus hippoglossus)

Halibut liver oil is now produced on a large scale and is highly valued because it can be retailed with a potency some 60 times greater in respect of vitamin A than average medicinal cod liver oil. In order to maintain such a high potency it is necessary to blend rich oils with poorer oils since the vitamin content of halibut livers exhibits marked seasonal fluctuations [Lovern *et al.* 1933]. The richer oils are consequently in great demand.

It is shown in the present study that in addition to the liver, the alimentary tract of the halibut provides a notable and hitherto neglected source of vitamin A. The stomach is practically devoid of vitamin, but the pyloric caeca and intestines, though not rich in fat, contain very large quantities of vitamin A. If, therefore, the viscera are worked up by the ordinary methods of fat extraction, very potent oils (up to 33% vitamin A) can be obtained. The halibut possesses four pyloric appendages which are comparable with the intestine in size and weight. The spleen is relatively large and contains a substantial quantity of vitamin whilst the mesentery is a by no means negligible source (*vide* Table XV).

The first series of experiments concerns the distribution of vitamin A between the liver and the "viscera", i.e. stomach, spleen, intestines, mesentery etc., worked up together. The second series eliminates the stomach as a source and shows that most of the "non-liver" vitamin A occurs in the pyloric caeca and intestines. The third series carries the distribution into the different portions of the intestines. Work is well advanced on the distribution in the epithelial, areolar and muscle tissues in the intestine.

Series 1. Eight fish (A–H) were caught in Shetland waters in May 1937 by the crew of the research vessel (Torry Research Station). The three smallest fish (F, G, H) were treated as one and labelled I.

The "pluck" or total viscera from each fish was divided into two portions, liver and other viscera, and worked up separately for oil. 12 samples were thus obtained: liver oils A–E and I, "visceral" oils A–E and I.

Table XV shows (*a*) the variability of the livers in respect of both fat and vitamin A, (*b*) the variability of the fat and vitamin A content of the "viscera", (*c*) that the amount of vitamin in the "viscera" is of the same order as that in the liver and (*d*) the high potency of the "visceral" oils.

Table XV. *Distribution of vitamin A in halibut*

	Series 1					
Fish ...	A	B	C	D	E	I
Wt. fish kg.	45·4	45·4	28·6	23·6	26·9	36·3
liver g.	1030	540	400	340	510	910
liver oil g.	203	70	34	—	—	205
vitamin A in liver oil g.	3·84	1·53	2·1	—	—	0·22
% vitamin A in liver oil	2·0	2·2	6·3	4·6	0·33	0·11
Wt. viscera g.	1670	1470	760	850	1040	1060
oil from viscera g.	36	27	15	15	17	20
vitamin A in oil g.	2·88	1·51	1·05	0·9	0·48	0·32
% vitamin A in oil	8·0	5·6	7·0	6·0	2·8	1·6

	Series 2	
Fish ...	A′	B′
Wt. viscera g.	3050	2450
oil from viscera g.	45·5	31·2
vitamin A g.	4·13	4·003
% vitamin A in oil	9·1	12·62
Wt. stomach g.	2073	1784
oil from stomach g.	11·74	11·06
Vitamin A	Negligible	
Wt. mesentery g.	207	155
oil from mesentery g.	5·08	3·8
vitamin A mesentery g.	0·055	0·085
% vitamin A in oil	1·2	2·2
Wt. spleen g.	63	45
oil from spleen g.	1·52	1·02
vitamin A in oil g.	0·007	0·018
% vitamin A in oil	0·45	1·8
Wt. intestines g.	707	467
oil from intestines g.	27·15	15·32
vitamin A in oil g.	4·07	3·90
% vitamin A in oil	15	25·5

	Series 3	
Fish ...	C′	D′
Wt. intestines g.	688	573
oil from intestines g.	33·65	27·28
vitamin A in oil g.	9·22	6·87
% vitamin A in oil	27·4	25·2
Wt. pyloric caeca g.	369	321
oil from pyloric caeca g.	22·6	16·7
vitamin A in oil g.	5·94	4·01
% vitamin A in oil	26	24
Wt. duodenum g.	112	116
oil from duodenum g.	4·67	6·61
vitamin A in oil g.	1·35	1·72
% vitamin A in oil	29	26
Wt. ileum g.	171	86
oil from ileum g.	6·12	3·41
vitamin A in oil g.	1·9	1·06
% vitamin A in oil	31·5	31·5
Wt. caecum g.	36	50
oil from caecum g.	0·26	0·56
vitamin A in oil g.	0·031	0·084
% vitamin A in oil	12	15

Series 2. Two large halibut A′ and B′ were used for determining the distribution of vitamin A in the alimentary tract. The livers were not used. The averaged results showed:

	% oil in wet tissue	% vitamin A in oil
Stomachs	*ca.* 0·6	Negligibly small
Mesenteries	2·5	1·6
Spleens	2·3	1·0
Intestines including pyloric caeca	3·3	19·0

In these fish some 98% of the "visceral" vitamin A was therefore localized in the intestines. Stated in another way, by working up the intestines only, 80% of the weight of viscera was discarded at the cost of losing half the fat and 1/50 of the vitamin. By rejecting the stomach only, 1/3 of the weight of material was retained, with 2/3 of the fat and nearly all the vitamin.

The "visceral" oil obtained in this way may contain 25% of vitamin A ($C_{20}H_{29}OH$) or about 50% of vitamin A ester (if it exists wholly as ester).

Series 3. The distribution in the intestines of two large halibut C′ and D′ was now determined. The pyloric caeca were extracted separately and the intestines were divided into three portions, two corresponding approximately with a duodenum and an ileum, and a third comprising the rectal portion carrying a cloacal caecum.

Very high potencies were again observed, with a very interesting distribution of the vitamin:

	% total intestinal vitamin A	Relative wt. of tissues expressed as % total wt. intestines
Pyloric caeca	61	55
Duodenum	18·6	18
Ileum	19·7	20
Rectal (cloacal) caecum	0·7	7

A rough parallelism obtains between weights of vitamin and tissue for the pyloric caeca, duodenum and ileum, but there is an abrupt fall in vitamin A content at the rectal end of the intestine. It is suggestive that in the stomach, which is said to be concerned with disintegration and digestion rather than absorption of food, there is no vitamin A, and that the terminal portion of the intestine, which is mainly concerned with excretion, is relatively poor in vitamin, whilst the intermediate parts of the alimentary tract are very rich.

The oils from the ileum appeared to contain 33–37 % of vitamin A when judged by the colour test and 31–32 % on the basis of ultraviolet absorption. This is a real discrepancy which will be investigated further.

Table XVI. *Spectroscopic data on oils from halibut viscera*

	Intestines		Pyloric caeca		Duodenum		Ileum	
$E^{1\%}_{1\,cm.}$ 693 mμ.	38	55	68	60	74	90	84	66
620	750	1280	1313	1200	1450	1280	1600	1872
583	410	650	687	650	745	774	832	953
325	240	406	440	373	460	360	500	500

Ratio $E^{1\%}_{1\,cm.}$ 693 mμ./$E^{1\%}_{1\,cm.}$ 620 mμ.

Livers	*ca.* 1 : 6
"Viscera" (stomach, mesentery intestines)	1 : 10 (mean)
Intestines only	*ca.* 1 : 20
Pyloric caeca	*ca.* 1 : 20
Duodena, ilea, cloacal caeca	1 : 14–1 : 28 (variable)

Table XVII. *Distribution of vitamin A in halibut viscera*

(*a*) Total vitamin A g.

Fish ...	A′	B′		C′	D′
Mesentery	0·055	0·085	Pyloric caeca	5·95	4·01
Spleen	0·007	0·018	Duodenum	1·35	1·72
Stomach	Nil	Nil	Ileum	1·9	1·06
Intestines	4·07	3·90	Cloacal caecum	0·03	0·084
Total	4·132	4·003			
Intestines account for	98·5 %	97·4 %			

(*b*) Wt. vitamin A/wet wt. tissues, p.p.m.

Liver	*ca.* 2500 possibly 500–10,000 in extreme cases
Stomach	Very low
Mesentery	400
Spleen	230
Pyloric caeca	14,420
Duodenum	13,410
Ileum	11,500
Cloacal caecum	1,340

Preliminary tests showed that by the time the material was available for study the vitamin A of the intestines was not confined to the innermost epithelial layers.

Discussion

(1) A satisfying interpretation of all the foregoing data is not yet possible. An attempt will be made to state the various problems clearly; this involves perhaps uncritical acceptance of the views of workers whose experience concerns experimental fields different from our own, but this may be unavoidable.

Granted that most of the larger fish are carnivorous, the mechanism of food assimilation in fishes will be primarily concerned with the metabolism of protein and fat rather than carbohydrate. The comparative poverty of some species in respect of vitamin A in the alimentary tract suggests that the substance is not a *sine qua non* for assimilation of either protein or fat. The richness of other species in vitamin, and the sharply selective distribution over the different regions of the alimentary tract, suggests on the other hand participation of vitamin A (possibly as an intermediary) in food assimilation.

It is known that in the eye vitamin A functions in loose association with protein, whilst its association with fat is too well known to need emphasis. The acceptance of the idea of plurality of functions seems inevitable for vitamin A.

Fat absorption

The physiology of fat digestion and absorption in fishes has been studied very little considering the importance of fat in the diet of most species. Among the more outstanding papers reference may be made to the work of van Herwerden [1908], Greene [1912, 1, 2; 1913] and Dawes [1930].

Van Herwerden studied mainly the gastric processes. He demonstrated the presence of active lipolytic enzymes in gastric mucosa and showed that the fat droplets normally occurring in stomach epithelia were absent from fasting fish.

Greene carried out histological studies on the fat-absorbing function of the alimentary tract of the King salmon (*Oncorhynchus tschawytscha*). The amount of fat taken up by the mucous lining membrane of the stomach is "quite sufficient to form a very striking picture" but is "not anywhere near so great in amount as that shown by the mucosa of other portions of the alimentary tract". The pyloric division of the stomach is much more active in fat absorption than the cardiac division, and in the pyloric caeca the process takes place abundantly. "Whatever else these organs accomplish, it is perfectly clear that absorption of fat is one of their chief functions." Fat is also readily absorbed by the intestinal epithelium of salmon. Greene pictures the fat components as diffusing through the free walls of columnar epithelial cells prior to resynthesis, and he finds that the role of the stomach in fat absorption tends to decrease with increasing age of the fish.

Dawes, working on plaice (*Pleuronectes platessa*), arrived at very similar conclusions. Absorption of fat in the stomach could be observed for the first food taken after fasting. When however the fish were feeding freely, the stomach played a minor part in fat absorption. The pyloric caeca were definitely regarded as sites of fat synthesis and absorption, the histological work showing the tips of the mucosal folds to be specially effective. The duodenal and intestinal epithelia showed no signs of fat if the fish had been fasting, but the cells were loaded with fat droplets when food was abundant. Even the rectum was found to be capable of some fat absorption.

Sinclair [1929], in his work on fat absorption in the mammal, adduces evidence to the effect that "within the epithelial cells of the intestinal mucosa, there is

Table XVIII. *Distribution of vitamin A in relation to sites of fat absorption*

Stomach	Fat absorption can occur to a minor degree in the normal state	Vitamin A is either absent from the stomach or present in amounts which are small in both a relative and an absolute sense
Pyloric caeca	Very definitely sites of fat synthesis and absorption [Greene; Dawes]	In many species the pyloric caeca contain a substantial amount of vitamin A which may be large in an absolute sense and is apparently always large in relation to the total resources of the entire fish
Intestines	Also very effective in fat absorption, at any rate in some species	Frequently rich in vitamin A
Rectal portion (cloacal caecum etc.)	Much less effective	Vitamin A, when present is in much smaller concentration than in other parts of the intestines

a 'specific' phospholipid which occupies an intermediary position between fatty acids and neutral fat

fatty acids $\rightleftharpoons$ phospholipid $\rightleftharpoons$ neutral fat",

and approximate constancy in the amount of this lipid is essential to his hypothesis [cf. Verzàr & Laszt, 1934, 1, 2].

Dawes reviewed his own studies in relation to Sinclair's views. He obtained negative tests for phospholipid under circumstances such that positive results might have been expected if Sinclair's hypothesis were valid for plaice. A negative histochemical test for phospholipid is inconclusive [Carleton, 1926] and Dawes' experience may be given in his own words:

"It may be that the technique does not favour detection of the 'specific' phospholipid or that the groups of molecules are too small to be visible under the highest powers of the microscope, but in any case it is not possible to present evidence which supports this (i.e. Sinclair's) hypothesis."

If it is fair to conclude that the phospholipid mechanism is not demonstrable for plaice, it is equally fair to conclude that a mechanism involving vitamin A is not demonstrably applicable to mammals. The paucity of vitamin A in the alimentary tract of the rabbit is in harmony with the work of Green [1934, 1, 2] on rats, wherein he showed that although vitamin A deficiency brings about a large decrease in the esterase content of blood serum, fat metabolism is not obviously altered, since absorption, mobilization, desaturation and oxidation of fats continue in the absence of vitamin A.

The distribution of vitamin A in the alimentary tract of many fishes suggests a mechanism akin to Sinclair's, with the vitamin playing the part of intermediary. The main difficulty with such an idea is the enormous disparity in the intestines of different species. Alternative mechanisms for fat absorption seem inescapable, unless the parallelism between vitamin A distribution and fat assimilation is fortuitous.

(2) In their comprehensive report on cod liver oil, Drummond & Hilditch [1930] discuss the origin of vitamin A in the liver:

"...results were somewhat surprising in that they revealed that the commonest foods of the cod were much poorer in these substances (vitamins A and D) than had been suspected. To what, then, are the relatively large stores of vitamin A normally found in the liver of the cod to be attributed? There are two alternative explanations that come to the mind. In the first place

there is the possibility that, although the amount of both vitamins in the daily food of the cod may be small, it may be retained in the liver so effectively that by the time the fish is mature the accumulation will be considerable. Alternatively there is the less attractive theory that the organism of the cod possesses the power to synthesize both vitamins A and D. For the time being, there is insufficient evidence to enable us to judge between these two theories, or to advance a more satisfactory one and further investigation is required to throw more light on the matter."

Later work [Drummond & Gunther, 1934] only served to increase the difficulties, for the zooplankton, an essential link in the hypothetical chain from the carotene of diatoms to the vitamin A of fishes, was repeatedly found to give negative or almost negative tests when fed to rats on a vitamin A-free diet, although diatoms gave positive results.

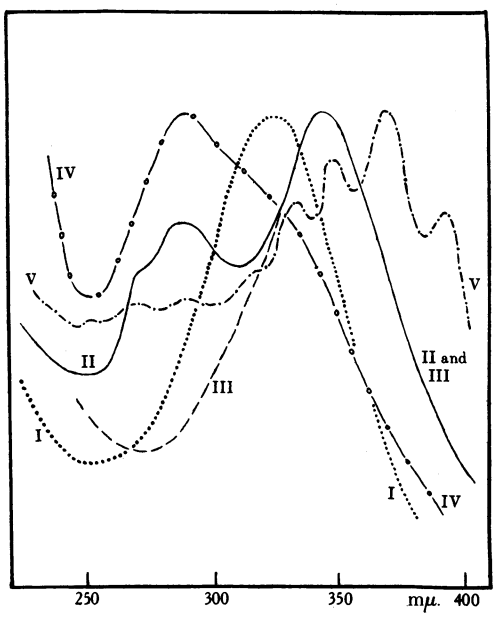

Fig. 2. I. Ultraviolet absorption spectrum of vitamin A; single maximum at 325 mμ. II. Typical ultraviolet spectrum of a material rich in factor A_2; the principal maximum is displaced progressively towards 350 mμ. as the ratio A_2/A increases. Note the subsidiary maximum at 285–290 mμ., and inflexion near 275 mμ. III. Fraction from II, soluble in 83% methyl alcohol. Selective absorption at 285–290 mμ. has been eliminated, without affecting the 350 mμ. band. IV. Fraction from II, insoluble in 83% methyl alcohol. The absorption is predominantly due to the 285–290 mμ. substance (A_3 ?). V. Cyclization product obtained by treatment of either I or II with $N/30$ alcoholic hydrogen chloride for 20 min. Narrow bands are seen with maxima at 392, 369, 350 and 333 mμ., and traces of ill-defined selective absorption further in the ultraviolet.

If the problem was regarded as acute for the cod, it is much more so for the halibut with its hundred-fold greater liver reserves [Lovern *et al.* 1933; Lovern & Sharp, 1933]. The discovery of large stores of vitamin A in the alimentary tracts of fishes complicates the position, for on the one hand it roughly doubles the recognized vitamin A intake in carnivorous fishes, and on the other hand it doubles the amount to be accounted for.

The contrast between for example a 4 cwt. sunfish and a large halibut is so striking as to render the claims of the "less attractive theory" more insistent.

(3) The vitamin A-like substances (i.e. polyene alcohols) present in the non-saponifiable extracts of oils, are evidently more numerous than is generally recognized:

Table XIX. *Polyene alcohols in non-saponifiable extracts of oils*

		Visible or ultraviolet maximum	$SbCl_3$ colour test maxima	Occurrence
Vitamin A, $C_{20}H_{29}OH$	Δ 5	325 mμ.	620 mμ. (605 mμ.) Blue colour	In eyes, liver, intestines, spleen, mesentery, etc. (*vide supra*)
Factor A_2	Δ 5	350 mμ. (288 mμ. ?)	693 mμ. Green colour	Do.
Chromogen(s)	Δ ?	?	635–640 mμ. 595 mμ. 557 mμ.	Sturgeon pancreatic and peritoneal fats, perch livers and viscera, trout viscera, mammalian blood?
Contaminant of mammalian "non-sap"	Δ 4 ?	286 mμ.	583 mμ.	Sheep liver, whale liver, some fishes
Chromogens	Δ ?	? Narrow bands 350–400 mμ. ?	660 mμ. 624 mμ.	Laboratory artefact(s)?
Acidic pigments (astacene type)	—	450–500 mμ.	Transient and feeble	Many zooplankton and some fishes

The above table is presented in order to assemble the known facts and to throw into relief the gaps in the evidence.

From the properties of the various chromogenic constituents, it is probable that the substances are polyene alcohols differing mainly in the number of conjugated double bonds. The fission *in vitro* of carotenoids at particular double bonds has eluded organic chemists; this is the more unfortunate since the properties of vitamin A analogues with one —CH=CH— more or less in the side chain might well clarify the whole problem of the natural chromogens.

The evidence now available suggests, but does not prove, that the 286 mμ. contaminant has one —CH=CH— group less than vitamin A and factor A_2 one more. The appreciably lower stability of factor A_2 to ultraviolet radiation is consistent with this hypothesis.

Summary

Whole eyes from a number of species of fish contain 3–7 p.p.m. vitamin A. Goldfish eyes are much richer and contain also the factor A_2 as shown by the 693 mμ. band in the colour test and the 345–350 mμ. maximum in the ultraviolet. Factor A_2 tends to replace vitamin A in freshwater fishes.

In the rabbit, the liver, with *ca.* 70 p.p.m., is the main vitamin A depot. Only traces were found in other organs and factor A_2 was not detected.

The herring contains relatively large quantities of vitamin A in the alimentary tract, particularly in the pyloric caeca. The latter may yield oil richer in vitamin A than cod liver oil.

Salmon pyloric caeca may contain more vitamin A than the livers of the same fishes. The non-saponifiable matter from the livers of frozen salmon contained 6% vitamin A and *ca.* 6% factor A_2, whereas a similar fraction from the pyloric caeca yielded 13% vitamin A and 1·3% factor A_2. The stomach contained only traces of vitamin A.

Trout also contain vitamin A and factor A_2 in the liver, pyloric caeca and intestines. Factor A_2 predominates over vitamin A in the brown trout and in the rainbow trout the ratio factor A_2/vitamin A is highest in the liver. The alimentary tract (especially the pyloric caeca) contains more vitamin than the liver.

Perch also contain vitamin A and its congeners at sites other than the liver.

Turbot contain little vitamin A (6–7 p.p.m.) in the stomach, but the remainder of the alimentary tract is relatively rich.

The conger eel stores about half its stock of vitamin A in the liver, the other half, apart from traces distributed widely, occurring in the body fat and the adipose tissue of the gonads.

Two large fish, one a spotted ray and the other a sunfish, contained surprisingly little vitamin A at any site.

In the cod, the pyloric caeca yield a few per cent of oil, the potency of which may be 10–20 times greater than that of the average cod liver oil.

The halibut has no vitamin A in the stomach but contains a very large quantity in the pyloric caeca and the intestines. Some intestinal oils contain 60–70 % of vitamin A esters. The distribution of vitamin A in halibut intestines runs parallel with the weight of tissue except in the post-absorptive rectal region.

The possibility that vitamin A participates in the process of fat exchange or assimilation is considered in relation to published histological studies, and the significance of vitamin A in the alimentary tract is discussed in relation to its origin and its function; various difficulties are pointed out.

The position concerning vitamin A congeners is reviewed with particular reference to the 693mμ. chromogen (factor A_2). The available information suggests strongly that it is a polyene alcohol akin to, but more unsaturated than, vitamin A. The distribution of factor A_2 is consistent with the sharing of some at least of the functions of vitamin A in fishes generally and fish from fresh water more especially.

We are indebted to the Medical Research Council for a grant to one of us (J. R. E.). Dr R. J. Daniel (Dept. of Oceanography, University of Liverpool), Mr H. T. Matthews, F.R.C.V.S. (Chief Veterinary Officer for Liverpool), and the Staff of the Marine Biological Station, Port Erin, have given valuable assistance. Mr R. H. Creed of the Spectroscopy Laboratory has assisted in the vitamin A tests, and Dr T. F. Hewer of the Dept. of Pathology kindly furnished us with post mortem material.

REFERENCES

Batty, Burawoy, Heilbron, Jones & Lowe (1937). *J. chem. Soc.* 755.
Capper (1930, 1). *Biochem. J.* **24**, 453.
—— (1930, 2). *Biochem. J.* **24**, 980.
—— McKibbin & Prentice (1931). *Biochem. J.* **25**, 265.
Carleton (1926). Histological Technique, p. 167. (Oxford Univ. Press: Humphrey Milford.)
Carr & Jewell (1933). *Nature, Lond.*, **131**, 92.
Dawes (1930). *J. Mar. biol. Ass. U.K.* **17**, 75.
Davies, Heilbron, Jones & Lowe (1935). *J. chem. Soc.* 584.
Drummond & Gunther (1934). *J. exp. Biol.* **11**, 203.
—— & Hilditch (1930). *Rep. Emp. Mktg Bd.* **35**.
—— & Macwalter (1933). *Biochem. J.* **27**, 1342.
—— —— (1934). *J. Physiol.* **83**, 236.

Drummond, Ahmad & Morton (1930). *J. Soc. chem. Ind., Lond.*, **40**, 291.
—— Gilding & Macwalter (1934). *J. Physiol.* **82**, 75.
Edisbury, Gillam, Heilbron & Morton (1932). *Biochem. J.* **26**, 1165.
—— Lovern & Morton (1937). *Biochem. J.* **31**, 416.
—— Morton & Simpkins (1937). *Nature, Lond.*, **140**, 234.
Euler, Euler & Hellström (1928). *Biochem. Z.* **203**, 370.
—— —— & Karrer (1929). *Helv. chim. Acta*, **12**, 278.
—— Karrer & Rydbom (1929). *S.B. Akad. Wiss. Wien*, B, **62**, 2445.
Green (1934, 1). *Biochem. J.* **28**, 16.
—— (1934, 2). *Biochem. J.* **28**, 25.
Greene (1912, 1). *Amer. J. Physiol.* **30**, 278.
—— (1912, 2). *Bull. U.S. Bur. Fish.* **32**, 73.
—— (1913). *Bull. U.S. Bur. Fish.* **33**, 149.
Heilbron, Gillam & Morton (1931). *Biochem. J.* **25**, 1352.
—— Heslop, Morton & Webster (1932). *Biochem. J.* **26**, 1178.
—— Gillam, Lederer & Rosanova (1937). *Nature, Lond.*, **140**, 233.
—— Jones, Lowe & Wright (1936). *J. chem. Soc.* 561.
v. Herwerden (1908). *J. phys. Chem.* **56**, 453.
Hume (1937). *Nature, Lond.*, **139**, 467.
Jeans & Zentmire (1934). *J. Amer. med. Ass.* **102**, 892.
Kuhn & Morris (1937). *S.B. Akad. Wiss. Wien*, **70**, 853.
Lederer & Rosanova (1937). *Biochemie*, **2**, 293.
Lovern & Sharp (1933). *Biochem. J.* **27**, 1470.
—— Creed & Morton (1931). *Biochem. J.* **25**, 1341.
—— Edisbury & Morton (1933). *Biochem. J.* **27**, 1461.
—— —— —— (1937). *Nature, Lond.*, **140**, 276.
Medical Research Council (1935). Special Report, No. 202.
Mellanby (1934). Nutrition and Disease; the interaction of Clinical and Experimental Work. (Edinburgh.)
Moore (1929, 1). *Lancet*, **216**, 499.
—— (1929, 2). *Biochem. J.* **23**, 803.
—— (1929, 3). *Biochem. J.* **23**, 1267.
Morgan, Edisbury & Morton (1935). *Biochem. J.* **29**, 1645.
—— (1935). The Application of Absorption Spectra to Vitamins and Hormones (Hilger).
—— (1937). V^{e} Congrès International Technique et Chimique Scheveningue. C. r. **1**, 58.
—— Heilbron & Thompson (1931). *Biochem. J.* **25**, 20.
Pritchard, Wilkinson, Edisbury & Morton (1937). *Biochem. J.* **31**, 258.
Rea & Drummond (1932). *Z. Vitaminforsch.* **1**, 177.
Rosenheim & Webster (1927). *Nature, Lond.*, **120**, 440.
Shorland (1937). *Nature, Lond.*, **140**, 223.
Sinclair (1929). *J. biol. Chem.* **82**, 1117.
—— (1933). *Physiol. Rev.* **14**, 351.
Verzàr & Laszt (1934, 1). *Biochem. Z.* **270**, 24.
—— —— (1934, 2). *Biochem. Z.* **270**, 35.
Wald (1934). *Nature, Lond.*, **134**, 65.
—— (1935, 1). *Nature, Lond.*, **136**, 832.
—— (1935, 2). *Nature, Lond.*, **136**, 913.
—— (1935, 3). *J. gen. Physiol.* **19**, 351.
—— (1936, 1). *J. gen. Physiol.* **19**, 781.
—— (1936, 2). *J. gen. Physiol.* **20**, 45.
—— (1937, 1). *Nature, Lond.*, **139**, 1017.
—— (1937, 2). *Nature, Lond.*, **140**, 197.
Wolff, Overhoff & Eekelen (1930). *Dtsch. med. Wschr.* **56**, 1428.
Woolf & Moore (1932). *Lancet*, **223**, 13.