CXCVII. THE VITAMIN A₁ AND A₂ CONTENTS OF MAMMALIAN AND OTHER ANIMAL LIVERS

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(Received 21 July 1938)

THE liver fats of animals in general rank amongst the richest sources of the growth-promoting and anti-xerophthalmic substance known as vitamin A which, in addition to its biological activity, is characterized by an ultra-violet absorption band near 328 m μ and by the blue colour that it gives with antimony trichloride in chloroform (main absorption maximum at 620 m μ). These properties of the vitamin are shown with slight modification by the liver fats both of marine fish and of mammals. When, however, the first preparations of really potent concentrates of the vitamin were obtained [Karrer *et al.* 1931; Heilbron *et al.* 1932] they were obviously heterogeneous, notably because they contained small but variable quantities of a substance exhibiting an absorption band at 693 m μ in the SbCl₃ blue colour [Heilbron *et al.* 1932]. It has since been observed that the liver fats of certain Russian freshwater fish contain more of the 693 m μ chromogen than of the more common one characterized by a 620 m μ band [Lederer & Rosanova, 1937] and more detailed spectrophotometric examinations of the liver oils of a variety of freshwater fish [Lederer *et al.* 1937; Edisbury *et al.* 1937; Gillam *et al.* 1938; Edisbury *et al.* 1938] have confirmed this. The same investigations have also shown that the ultra-violet absorption spectra of the oils are characterized by two maxima near 280 and 350 m μ , respectively, instead of the single maximum at 328 m μ common to the liver oils of marine fish.

It has been suggested that the 693 m μ chromogen might be a second vitamin A, possibly specific to freshwater fish [Lederer *et al.* 1937], whilst Edisbury *et al.* have tentatively named it vitamin A₂ [1937] and later "factor A₂" [1938]. Although in our earlier concentration of the substance it was not found possible to separate it completely from vitamin A₁ the results of feeding experiments on rats [Gillam *et al.* 1938] indicate that it actually possesses the biological activity always associated with vitamin A₁, whilst the chemical evidence suggests that it is probably a higher isologue (C₂₂H₃₂O) of this vitamin which, itself, has been shown to have the formula C₂₀H₃₀O [Karrer *et al.* 1931; Heilbron *et al.* 1932]. More recent experiments with the physiologically potent concentrate previously examined have shown that it contains another chromogen (645–650 m μ) the presence of which makes the 620 m μ chromogen value too high [Lederer, 1938]. On the assumption that this chromogen has no vitamin A activity itself, Lederer [1938] draws the conclusion that more of the total activity of the concentrate must be due to the 693 m μ chromogen than was previously supposed. This further justifies the title vitamin A₂, as also does the fact found by Wald [1937, 1, 2] that the 693 m μ chromogen can replace vitamin A₁ in the visual cycle that occurs in the retinae of freshwater fish.

The generalization that is beginning to emerge from these results is that whereas marine fish liver oils contain the 620 m μ chromogen (vitamin A or A₁) with only traces of the 693 m μ chromogen (vitamin A₂),¹ freshwater fish liver oils

tend to contain more vitamin A₂ than vitamin A₁, or at least a larger proportion of vitamin A₂ than do the marine fish liver oils. The only mammals that have been examined for vitamin A₂, namely, the rabbit [Edisbury *et al.* 1938] and the whale (private communication from Dr R. A. Morton), apparently contain none of this substance.

The present paper records the results obtained by the examination of the livers of a series of animals and birds with particular reference to the total vitamin A content of the liver and the presence or absence of vitamin A₂.

EXPERIMENTAL

The procedure adopted in all cases was, firstly, hot saponification of the fresh liver with KOH (2 ml. 10 % KOH in 50 % aqueous alcohol per g. of fresh liver: time of saponification 1–3 hr.) until the tissue was completely broken down. After cooling, the mixture was diluted with water and extracted with ether, the resulting extract being washed with water, dried over anhydrous Na₂SO₄ and evaporated to dryness in N₂. Part of the unsaponifiable matter so obtained was treated with SbCl₃ in chloroform, the blue colour being examined spectroscopically by methods already described [Heilbron *et al.* 1931]. Values for the relative amounts of vitamins A₁ and A₂ present were obtained from the ratio of the intensities of the absorption bands at 620 and 693 mμ, respectively, of the blue solution. The remaining unsaponifiable matter in chloroform was diluted 10–20 times with alcohol and its ultra-violet absorption spectrum determined photographically, using a Hilger E3 quartz spectrograph. The vitamin A content was taken as the mean of the two values obtained from the intensity of absorption of the alcoholic and chloroformic SbCl₃ solutions, respectively, on the provisional basis for pure vitamin A of $E_{1\%}^{1\text{cm}} \frac{620\text{ m}\mu}{328\text{ m}\mu} = 5000 \text{ (SbCl}_3\text{)}$ [Carr & Jewell, 1933].

The results are recorded in Table I.

DISCUSSION

Consideration of the data and of previous work [cf. Kuhn, 1933] leads to the conclusion that in marine fish in general (or possibly in some of the animals on which they feed) carotene is ingested (either directly or indirectly from the phytoplankton) and the C₄₀H₅₆ molecule is broken down into C₂₀ fragments constituting the typical vitamin A. The small amount of vitamin A₂ normally present would appear either to be produced as a by-product of this process in the fish themselves, or to be received by them preformed to this small extent from the lower animals on which they feed. On the other hand, freshwater fish (or again some lower organisms eaten by them) must be able, in addition, to break down the carotene molecule into C₂₂ and C₁₈ fragments, the C₂₂ fragment, at least, appearing in the liver in greater concentration than is normally met with in marine fish livers.

The complete absence of vitamin A₂ from the livers of the majority of both the individuals and the species of mammals and birds examined indicates that

¹ Karrer has suggested the name "Axirophthol" for the classical vitamin A and the class name "Axirophthols" for the higher isologues [Euler *et al.* 1938]. Using Karrer's nomenclature, the particular axerophthol (C₂₂H₃₂O) which our earlier experiments indicate as the 693 mμ chromogen would be called β-*apo*-5-carotinol. Karrer and his collaborators have prepared what they believe to be this compound and whilst it gives a blue colour with SbCl₃ the position of the absorption band that gives rise to the colour is apparently quite different from 693 mμ. If the formula of the compound is beyond doubt then the formula suggested for the 693 mμ chromogen will need modification.

Table I

Animal	Source, etc.	Wt. fresh liver g.	Estimated total vitamin A mg.	Vitamin A mg. per 100 g. liver	693 m μ chromogen	Ratio of intensities 693/620 m μ
Lion <i>Felis leo</i>	Zoological Gardens (20 yr. old)	2000	160	8.0	Absent	0.057
Gibbon <i>Hylobates concolor</i>	Zoo (6 months old)	110	0.75	0.70	„	Not measurable
Giant monitor <i>Varanus salvator</i>	Zoo	(a) 200 (b) 113	500 74	250 65	Present —	0.11 —
Python <i>Python reticulatus</i>	Zoo (very old animal)	800	688	86	Absent	0.023
Alligator <i>Alligator mississippiensis</i>		225	8.0	3.5	„	<0.20
Rabbit <i>Lepus cuniculus</i>	Wild	27.6	4.75	17.2	„	0.073
Rat <i>Mus decumanus</i>	Wild town animal, ♂	9.2	0.55	6.0	„	0.031
Mouse <i>Mus musculus</i>	Laboratory animals (a) Av. of 9 (b) Av. of 15	1.63 1.14	0.013 0.0125	1.1 1.1	„ „	Not measurable —
Guinea-pig <i>Cavia porcellus</i>	Laboratory animals Av. of 3	10.4	0.07	0.68	„	0.076
Dog		278	3.06	1.1	„	<0.20
Cat		66.5	2.4	3.6	„	0.033
Ox		5235	340	6.5	„	0.052
Cow		6075	132	2.17	„	0.082
Lamb		(a) 506 (b) 280	56 51	11.0 14.7	„ „	0.058 0.063
Pig		(a) 844 (b) 1060	67 10	7.94 1.0	„ „	0.044 —
Frog <i>Rana temporaria</i>	Local, av. of 6	0.41	Order of 0.0043	1.04	„	Not significant
White Whale <i>Beluga leucas</i>		—	—	—	„	0.066
Hen	Av. of 2	28	3.4	12.1	„	0.068
Duck	Av. of 4	24	2.93	12.2	„	<0.14
Turkey		(a) 118 (b) 128	24.7 26.4	21.0 20.7	„ „	0.044 0.06
Pigeon	Av. of two young birds	4.2	1.37	32.5	„	0.10
Kingfisher <i>Alcedo ispida</i>	Cheshire (one young fledgling: found dead)	1.9	0.15	7.7	Not detected	—
Seal <i>Phoca vitulina</i>	Russia	—	—	—	Present	0.20
Otter <i>Lutra vulgaris</i>	Cumberland	191	90	47	„	0.22
Rat (fed on vitamin A ₂ concentrate)		—	—	—	„	1.40

they cannot convert carotene directly into vitamin A₂ for, in the herbivorous animals at least, there can be no doubt that the diet normally contains relatively large quantities of carotene. The question arises, however, whether mammals can actually pass preformed vitamin A₂ into the liver when it is taken into the digestive tract. To test this point Dr Lederer very kindly supplied us with extracts of the livers of rats fed on concentrates of vitamin A₂. Examination of these extracts (cf. Table I) revealed the presence of relatively large quantities of the 693 m μ chromogen; in fact the livers of the rats were, chromogenically, very similar to those of freshwater fish.

Since mammals normally do not appear to manufacture the 693 m μ chromogen but can pass it into the liver when it is contained in the food eaten, it was argued that mammals that feed on fish should store some of this substance in their livers. Interesting test cases of this point are provided by the seal, for a liver extract of which we are indebted to Dr Lederer, and the otter, of the liver of which a sample was obtained from Cumberland through the kindness of Dr W. S. M. Grieve. Examination showed that both these livers contained vitamin A₂ in easily detectable amounts (cf. Table I). On the other hand the only specimen of a bird feeding on freshwater fish that we could obtain (i.e. the kingfisher) was found to have no detectable vitamin A₂ in its liver¹ (cf. Table I). Thus of all the mammals examined the only ones having the 693 m μ chromogen present in the liver were those known to feed on, or to have fed upon, fish. Of the remaining animals and birds examined the only other having any detectable quantity of this chromogen was the giant monitor in which, however, the proportion of this substance present was very small (cf. Table I).

It is interesting to note that the python and the giant monitor had the largest total weight of vitamin A₁ per liver of all the species examined; moreover, on the basis of percentage weight of vitamin in the liver, these two species stand alone. The particular python examined was a very old 25 ft. specimen and it may be that, as in the case of the halibut where the vitamin content of the liver increases with age, individuals of this species also accumulate vitamin A as they become older. Of the remaining species examined with the exception of the otter the percentage of vitamin A in the liver only varied within relatively narrow limits; thus in 18 species the average was 8.3 mg. per 100 g. fresh liver, whilst the limits of variation were of the order 1 to 32 mg. per 100 g. When mammals are compared with fishes much larger variations are, however, observed [cf. Edisbury *et al.* 1938].

In this preliminary survey only a few individuals of each species have been examined but it is probable that if more were examined much wider variations would be found. On the other hand the work of Davies & Moore [1935] indicates that the level of vitamin A in the liver of the rat usually falls within narrow limits; these workers have shown that when rats are fed with very high dosages of vitamin A the abnormally high resultant value for the vitamin content of the liver rapidly falls to normal as soon as the vitamin intake is reduced.

The differences between freshwater fish, marine fish, mammals and birds in the reaction of their liver extracts towards SbCl₃ are summarized in Table II.

Table II. *Summarized data on the relative amounts of 693 and 620 m μ chromogens in the livers of fish, animals and birds*

	No. of species included*	Ratio of the intensities of absorption at 693/620 m μ		693 m μ absorption band
		Range found	Average	
Freshwater fish†	11	0.25-2.62	1.680	Usually strongly positive
Marine fish†	11	0.04-0.17	0.110	Normally just detectable
Animals (almost all mammals)	11	0.03-0.17	0.060‡	Only rarely present
Birds	5	0.04-0.10	0.068‡	Only rarely present

* Some of these species have been represented by many specimens, others by only one.

† Cf. Gillam *et al.* [1938] and Lederer [1938] for some detailed examples.

‡ These values, which might be expected to be zero in the absence of the 693 m μ band, probably represent the overlap of the 693 m μ band at 620 m μ .

¹ The author is indebted to Miss Legge of the Manchester Museum for this specimen.

SUMMARY

Extracts of the livers of a number of species of animals and birds have been examined spectroscopically with particular reference to their contents of vitamins A_1 and A_2 .

It has been found that, in general, mammal and bird livers contain vitamin A_1 but no vitamin A_2 . In the 21 species examined the only exceptions found were the giant monitor (considerable vitamin A_1 and only traces of vitamin A_2) and animals known to feed on fish, i.e. the seal and the otter. A mammalian liver exceptional in that it contained more vitamin A_2 than vitamin A_1 was obtained by feeding a rat with a concentrate of freshwater fish liver. It would thus appear that although mammals cannot metabolize vitamin A_2 they can pass it into the liver if they receive it preformed into their digestive tract.

The vitamin A_1 contents of all the livers examined fell within the rather narrow limits of 0.7–32 mg. per 100 g. fresh liver (average for 18 species = 8.3 mg. per 100 g.) with the exception only of the python, giant monitor and otter with values of 86, 250 and 47 respectively.

The author is indebted to Prof. I. M. Heilbron for his interest in this work and to Dr J. F. Wilkinson and Mr G. Iles, Curator of the Belle Vue Zoological Gardens, Manchester, for specimens of the livers of certain animals. He also owes thanks to Dr R. W. Fairbrother, Dr T. H. B. Bedford and Dr W. Schlapp of this University for specimens of other animals' livers.

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