Low glucose values seem to parallel relatively high rates of glycolysis and relatively low vascularization, although spleen is again an exception. Gibson *et al.* (1946) assessed the blood content of dog brain and muscle at about 2% of the tissue weight, compared with 20% in kidney, lung and liver.

The glucose concentration as measured is the resultant of the concentrations in the different tissue spaces. The low glucose values for brain and testis suggest that the glucose concentration of the 'extracellular' space in these tissues is lower than that of plasma. As the size of the 'glucose space' is not known no precise calculations can be made.

The glucose content increased after death in liver and muscle, tissues known to contain appreciable amounts of glycogen, but fell in spleen, kidney and lung. The level of reducing substances other than glucose remained steady after death in those tissues which showed a relatively high initial value (brain, testis, muscle) and increased where the initial value was low (spleen, kidney, lung and liver).

SUMMARY

1. The true glucose content of eight rat tissues has been measured. Glucose was separated by paper chromatography and determined by the method of Nelson. The total reducing value was also determined.

2. When rats were killed by immersion in liquid oxygen, all the tissues except liver contained less glucose than did plasma, the value for brain and testis being only about one-tenth, and for muscle and spleen about a quarter, of the value for plasma.

3. The effects of delays in freezing the tissue after death were measured.

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Vitamin A₂ in Indian Fresh-water Fish-Liver Oils

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The possible existence of a new chromogen showing an absorption band at 693 m μ . in the colour test with antimony trichloride was first postulated by Heilbron, Gillam & Morton (1931). A little later, Edisbury, Morton & Simpkins (1937) designated this new chromogen vitamin A₂, on the strength of the very close similarity in the distribution of

vitamin A₁ (620 m μ . band, colour test) in the liver oils of marine fishes and of the new chromogen in most fresh-water fishes. Simultaneously, Lederer & Rosanova (1937), in an examination of liver oils from fishes of Russian rivers, observed the predominance of the 693 m μ . band over the 620 m μ . band of vitamin A₁. Its importance in the visual

cycle of retinas of fresh-water fishes was recognized by the work of Wald (1937). The occurrence of vitamin A₂ in small amounts has now been shown in many oils from marine sources [e.g. Morton & Stubbs, 1948; Cama, Collins & Morton, 1951 (European waters) and Balasundaram, Cama, Sundaresan & Varma, 1956 (Indian waters)] and also to some extent in amphibia (Collins, Love & Morton, 1953).

After much earlier confusion, the elucidation of the structure of vitamin A₂ as the dehydro-vitamin A₁ formula was proved conclusively by synthesis (Farrar, Hamlet, Henbest & Jones, 1952). The dehydro-vitamin A₁ formula was suggested earlier by Morton, Salah & Stubbs (1947), mainly on the basis of the properties of crystalline vitamin A₂ aldehyde (retinene₂). Very recently an elegant conversion of vitamin A₁ into vitamin A₂ has been successfully carried out (Henbest, Jones & Owen, 1955) which makes the latter readily accessible. The aldehyde of vitamin A₁ (retinene₁) is treated with *N*-bromosuccinimide to obtain retinene₂, which on reduction with lithium aluminium hydride yields vitamin A₂.

Owing to its labile nature and, in part, to the scarcity of high-potency liver oils from fresh-water fishes, vitamin A₂ has not yet been obtained in a crystalline form. The spectroscopic properties (Shantz, 1948; Cama & Morton, 1953) and the biological potency of vitamin A₂, which is approximately 40% of that of vitamin A₁ (Shantz & Brinkman, 1950), have been determined with the purest preparation obtained.

Data on the content of vitamins A₁ and A₂ of liver oils from Indian fresh-water fishes measured spectrophotometrically are not available, and not much is known of the relative distribution of the vitamin. The present paper gives the result of investigations carried out on fishes in large fresh-water reservoirs in the States of Mysore and Madras. A short report of the work has already been published (Balasundaram, Cama, Sundaresan & Varma, 1955*a, b*).

EXPERIMENTAL

Fish-liver oils. Livers from fresh-water fishes were mainly obtained from the River Cauvery in the States of Mysore and Madras. A few samples were also obtained from Lake Hassargatta (Bangalore). The dietary habits of the fishes were either predatory (fish eating other fishes), or were based on subsistence on algae and vegetation. The two groups of feeders are marked (X) and (Y) in Table 1.

Potassium chromate and potassium dichromate. These were of Merck and AnalaR quality respectively, and were used to calibrate the Beckman spectrophotometer.

Solvent. *cyclo*Hexane, for spectroscopic use, was obtained from B.D.H. Ltd.

Light petroleum (b.p. 40–60°). This material, obtained from Burmah-Shell, was allowed to stand over KMnO₄

for a few days, washed, dried over CaCl₂ and twice distilled before use.

Chloroform (B.P.). This was allowed to stand over CaCl₂ and was filtered before use to remove any traces of ethanol.

Diethyl ether. This was allowed to stand over Na wire and freshly distilled before use over reduced Fe to remove any peroxide.

Alumina for adsorption chromatography. Alumina, specially prepared for chromatographic investigations (Merck), was used by weakening with known amounts of water, usually 5% (v/w), which was stirred in slowly under light petroleum.

Reagents. SbCl₃, acetic anhydride and KOH were B.D.H. laboratory reagents. Anhydrous reagent-grade Na₂SO₄ was generally used.

Micrometer syringe. For quantitative colour-test determinations, portions were accurately measured with the Agla micrometer syringe (Burroughs Wellcome Ltd.).

Absorption spectra and colour-test determination. The ultraviolet spectra of solutions were determined with the Beckman spectrophotometer, and in the absence of a Hilger-Nutting visual spectrophotometer antimony trichloride colour tests at specific wavelengths were done with the same instrument.

Calibration of the spectrophotometer. The spectrophotometer was calibrated with potassium chromate and dichromate (Cama *et al.* 1951).

Extraction and assay of oil. Fresh livers were ground with acid-washed sand and anhydrous sodium sulphate, and the oil was extracted with light petroleum (b.p. 40–60°). The combined extracts were reduced in bulk at low pressure and the ultraviolet spectra on the whole oil were obtained. A portion of the oil was saponified, and absorption spectra determinations and colour tests at 693 and 620 mμ. were carried out.

RESULTS

The contribution of vitamin A₂, in the ultraviolet spectrum of vitamin A₁ at 325 mμ., is appreciable. On the other hand, in the antimony trichloride colour test of vitamin A₂ at 693 mμ., the contribution of vitamin A₁ at 693 mμ. is almost negligible. The colour test at 693 mμ. is thus specific for the estimation of vitamin A₂ on the unsaponifiable fractions of fish-liver oils, and the reading could be evaluated for the ultraviolet absorption of vitamin A₂ at 350 mμ. The colour test for vitamin A₂ shows absorption at 620 mμ. equal to that at 693 mμ. divided by 2.45. From these figures, vitamin A₁ could be estimated by the colour test at 620 and 693 mμ., after applying proper corrections, and the reading is correlated to the equivalent ultraviolet absorption at 325 mμ. The method of estimation of vitamins A₁ and A₂ illustrated by Cama & Morton (1953) was followed. Only in a few cases, vitamin A₁ was estimated by the three-point correction method of Cama *et al.* (1951). Table 1 shows the vitamin A₁ and A₂ contents of liver oils from different species of fresh-water fishes, and Fig. 1 shows typical vitamin A₂ spectra of two liver oils of high potency from fresh-water fishes, examined after saponification.

Table 1. Spectroscopic data from liver oils of some Indian fresh-water fishes

Solvent used was cyclohexane. X, predatory fish; Y, fish with vegetarian (algae) diet. $E_{1\text{cm}}^{1\%}$ values for pure substances in cyclohexane: crystalline vitamin A alcohol (326.5 m μ) = 1745; SbCl₃ colour test (620 m μ) = 5070 (Cama *et al.* 1951). Pure vitamin A₂ alcohol (351 m μ) = 1320; SbCl₃ colour test (693 m μ) = 3870 (Cama & Morton, 1953).

Reference	Name of species	Source	Percentage of oil from livers	λ_{max} of unsp. (m μ)	At 351 m μ vitamin A ₂	At 693 m μ vitamin A ₂	At 620 m μ vitamin A ₁	351 m μ vitamin A ₂	326.5 m μ vitamin A ₁	Corrected ($E_{1\text{cm}}^{1\%}$ *)
A	'Wallago attu' (X)	Mysore, Madras	4	290, 351	127	307	157	106	11	11
B	<i>Ophiocephalus striatus</i> (X)	Hessaraghatta	4	290, 351	265	582	311	200	25	25
C	<i>Ophiocephalus striatus</i> (X)	Mysore	4	285, 351	99	252	102	89	Nil	Nil
D	<i>Macropodus aor</i> (X)	Madras	6	290, 351	175.9	355	130	121	Nil	Nil
E	<i>Silundia species</i> (X)	Madras	5	287, 345	157	272	156	93	15	15
F	<i>Mystus species</i> (X)	Madras	5	290, 351	115.7	237	90	81	Nil	Nil
G	<i>Callichthys bimaculatus</i> (X)	Hessaraghatta	15	290, 345	259	618	329	211	26	26
H	<i>Ophiocephalus punctatus</i> (X)	Hessaraghatta	3	290, 345	44.1	62.7	40.6	21.4	5.1	5.1
I	<i>Ophiocephalus muraius</i> (X)	Madras	14	290, 330	53.1	86.5	44.5	29	3	3
J	<i>Nothopterus nolaopterus</i> (X)	Hessaraghatta	2	330	23	31.3	50.7	12.4	12.3	12.3
K	<i>Cirrhina cirrhosa</i> (Y)	Madras	—	330	18.2	15	30	10.7	19.7	19.7
L	<i>Calla calla</i> (Y)	Madras	5	330	8	11.1	7.8	5.1	8	8
M	<i>Pungasius species</i> (X)	Madras	2	275, 340	8	11.1	7.8	3.8	1.1	1.1
N	<i>Mastomembelus armatus</i> (Y)	Hessaraghatta	10	275, 287, 345	1.7	3.55	1.85	1.2	0.13	0.13
O	<i>Barbus carnaticus</i> (Y)	Mysore	4	330	8.13	7.8	—	2.78	6.95†	6.95†
P	<i>Barbus dubius</i> (Y)	Mysore	13	330	3.53	4.4	—	1.5	2.9†	2.9†
Q	<i>Labeo species</i> (Y)	Mysore	15	290, 335	7.47	9.8	—	3.35	3.6†	3.6†

* Expressed in terms of weight of original oil.
† Calculated by the three-point correction method (Cama *et al.* 1951).

Fig. 1. Absorption curves for unsaponifiable fractions of high-potency liver oils of fresh-water fishes. (Solvent: cyclohexane). ●, 'Wallago attu'; ○, *Ophiocephalus striatus*.

Chromatography of oils. A large number of fractions were collected by chromatography of oil (about 0.2 g.) on a column of weakened alumina. The details of the original procedure (Barua & Morton, 1949) were followed. Each of the 10–20 ml. fractions collected was examined on the spectrophotometer, and those showing identical maxima were combined and their $E_{1\text{cm}}^{1\%}$ values estimated. The results on two high-potency oils are recorded in Table 2.

Fraction I, obtained by chromatography of 'Wallago attu' oil (A), had marked absorption in the visible region of the spectrum. The fraction was reduced in volume and rechromatographed over alumina (without weakening). Distinct separation of a fine orange ring which travelled slowly down the chromatogram was effected; the pale greenish anhydro-vitamin A₁ with absorption at 350, 370 and 390 m μ . came down much faster. The orange ring had absorption maxima at 420, 450 and 470 m μ . characteristic of β -carotene, and behaved as such on the column.

Another interesting observation was the presence on the original column ('Wallago attu' oil) of a brownish-red band above the yellow vitamin A₂ alcohol band. This band was fast adsorbed on the column and did not move appreciably with light petroleum. The column was extruded and the band eluted with 50% ether-light petroleum mixture. When examined spectrophotometrically, the band had a main absorption maximum in the visible region at about 450 m μ ., characteristic of lutein (?). Further work on the purification and characterization of the carotenoids is in progress.

Table 2. *Spectroscopic data from fractions obtained after chromatography of 'Wallago attu' (A) and Ophiocephalus straitus (B) oils on 5% weakened alumina*

Fractions	Solvent	$\lambda_{\max.}$ (m μ .)	$E_{1\text{cm.}}^{1\%}$ 350 m μ .*		Component
			Oil A	Oil B	
1	Light petroleum (b.p. 40–60°)	350, 370, 390	0.121	0.101	Anhydro-vitamin A ₁
2	Light petroleum (b.p. 40–60°)	290, 336.7, 345	1.72	0.454	—
3	Light petroleum (b.p. 40–60°)	285, 350	86.80	184.8	Vitamin A ₂ esters
4	Ether–light petroleum (2:98, v/v)	330	14.0	14.6	Vitamin A ₁ alcohol
5	Ether–light petroleum (5:95, v/v)	330, 350, 370, 390	2.25	—	Anhydro-vitamin A ₂
6	Ether–light petroleum (10:90, v/v)	285, 350	12.65	17.0	Vitamin A ₂ alcohol
7	Ether–light petroleum (15:85, v/v)	330, 355, 370	0.933	0.42	—
8	Ether–light petroleum (20:80, v/v)	336.7, 350	0.844	—	—
9	Ether	330	1.81	4.41	Oxidized vitamins A ₁ & A ₂ (?)
Gross $E_{1\text{cm.}}^{1\%}$ (350 m μ .) of the whole oil			127.0	243.8	
Total $E_{1\text{cm.}}^{1\%}$ (350 m μ .) of the fractions			121.13	221.78	

* Expressed in terms of weight of original oil.

DISCUSSION

It is evident from Table 1 that different species of fresh-water fish-liver oils vary in vitamins A₁ and A₂ potency to a considerable extent. The vitamin A₂ content of some of the oils examined is very high relative to oils previously reported in literature (Edisbury, Morton, Simpkins & Lovern, 1938; Gillam, Heilbron, Jones & Lederer, 1938; Lederer & Verrier, 1939; Cama *et al.* 1952; and Abdullah, Morcos & Salah, 1954). The oils which show high amounts of vitamin A₂ and little of vitamin A₁ are from fish having predatory habits, whereas oils from fish subsisting on vegetation have invariably low amounts of vitamin A₂. In this respect *Notopterus notopterus* (J), which is comparatively a very small fish, rarely growing to more than 2 lb. in weight, is an exception. *Pungasius* species (M) is also low, for no obvious reason.

Chromatographic investigations on high-potency oils show that most of the vitamin A₂ in the oils is present in the ester form without the simultaneous presence of vitamin A₁ ester (presence of 693 m μ . band and absence of 620 m μ . band with the antimony trichloride colour test on the vitamin A₂ ester fraction). Vitamin A₁ is present in the alcohol form only, and a small amount of vitamin A₂ alcohol is also present (Table 2).

The presence of β -carotene and lutein (?) in 'Wallago attu' liver oil is of interest. Similar observation on the fresh-water fish, brown trout, has also been reported earlier by Steven (1948). The biogenesis of vitamin A₂ is obscure, but it may well be that β -carotene is the precursor, as suggested by Morton & Creed (1939).

SUMMARY

1. Spectrophotometric determination of vitamins A₁ and A₂ on some Indian fresh-water fish-liver oils have been carried out.

2. Some species of fresh-water fishes show very high amounts of vitamin A₂ in their liver oils.

3. Chromatographic investigations indicate that most of the vitamin A₂ is present in the ester form and a small amount of vitamin A₂ alcohol is also present. Vitamin A₁, whenever present, is in the alcohol form only.

4. The occurrence of small amounts of β -carotene and lutein (?) in liver oils of fresh-water fishes is of interest.

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Fungal Detoxication

THE METABOLISM OF ω -(2-NAPHTHYLOXY)-*n*-ALKYLCARBOXYLIC ACIDS BY *ASPERGILLUS NIGER*

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Detoxication studies in higher animals have been extensively reviewed by Williams (1947), who has used the term 'detoxication' to cover all drug metabolism, whether or not associated with a decrease in toxicity. Its importance in other biological systems is now becoming increasingly apparent, e.g. in the development of drug resistance in bacteria (Albert, 1951, p. 168), in selective weed control (Wain, 1954) and in insecticidal action (Casida, 1955). The metabolism of toxic materials by fungi, however, has received little attention except in a number of specific instances (Rich & Horsfall, 1954; Byrde & Fielding, 1955). In view of its fundamental importance in fungicidal action, we have now commenced a systematic study of fungal detoxication.

The fungicidal activity of certain ω -aryloxy-alkylcarboxylic acids (Crowdy, 1948) and the knowledge that they are capable of translocation in plants led to investigations of their possible value as systemic fungicides against plant disease (Crowdy & Wain, 1951; Byrde, Crowdy & Woodcock, 1953). Such studies were confined to assessments of their fungitoxicity both *in vivo* and *in vitro*, no attempt being made to investigate the biochemistry of the host-parasite-fungicide relationships.

From other sources, however, there is considerable evidence, both direct and indirect, that ω -aryloxyalkylcarboxylic acids undergo β -oxidation in plants, forming the corresponding aryloxyacetic acid or phenol according as the side chain possesses an even or an odd number of carbon atoms (Synerholm & Zimmerman, 1947; Fawcett, Ingram & Wain, 1954; Wain & Wightman, 1954; Luckwill &

Woodcock, 1955). It has also been suggested (Holley, Boyle & Hand, 1950) that nuclear hydroxylation (in an unspecified position) may occur during the metabolism of 2:4-dichlorophenoxyacetic acid in plants.

Little is known of the metabolism of such acids by micro-organisms, however, though ω -phenyl-substituted fatty acids have been studied (Rittenberg & Ivler, 1952; Webley, Duff & Farmer, 1955). It was demonstrated by Webley *et al.* (1955), who used *Nocardia opaca* Waksman & Henrici, that, although acids with an odd number of carbon atoms in the side chain underwent β -oxidation to benzoic acid, *o*-hydroxyphenylacetic acid was the only product identified from the even-numbered acids. They considered that this acid was formed by a side reaction from phenylacetic acid, the remainder of which was metabolized further. Evans & Smith (1954) isolated a small motile bacterium from soil, which converted 4-chlorophenoxyacetic acid into 2-hydroxy-4-chlorophenoxyacetic acid and 4-chlorocatechol. A small Gram-negative motile rod was also isolated, which converted 2:4-dichlorophenoxyacetic acid into a phenolic acid, presumed, on chromatographic evidence, to be 6-hydroxy-2:4-dichlorophenoxyacetic acid. Brown & McCall (1955), however, have reported that this metabolite is not 2:4-dichloro-6-, 4-chloro-2- or 2-chloro-4-hydroxyphenoxy acetic acid.

The investigations described in this and subsequent papers were undertaken to explore the metabolism of ω -(2-naphthylloxy)-*n*-alkylcarboxylic acids by fungi. Some of the results formed the basis of a preliminary communication (Woodcock & Byrde, 1955).