- Leplat, G. (1933a). *C.R. Soc. Biol., Paris*, **112**, 1256.
 Leplat, G. (1933b). *C.R. Ass. Anat.* **28**, 404.
 Lowry, O. H., Gilligan, D. R. & Katersky, E. M. (1941). *J. biol. Chem.* **139**, 795.
 Ma, T. S. & Zuazaga, G. (1942). *Ind. Engng Chem. (Anal.)* **14**, 280.
 McIlvaine, T. C. (1920). *J. biol. Chem.* **49**, 183.
 Manery, J. F. & Hastings, A. B. (1939). *J. biol. Chem.* **127**, 657.
 Nageotte, J. (1927a). *C.R. Soc. Biol., Paris*, **96**, 172.
 Nageotte, J. (1927b). *C.R. Soc. Biol., Paris*, **96**, 464.
 Nageotte, J. (1927c). *C.R. Soc. Biol., Paris*, **96**, 828.
 Nageotte, J. (1927d). *C.R. Soc. Biol., Paris*, **97**, 559.
 Nageotte, J. (1928). *C.R. Soc. Biol., Paris*, **98**, 15.
 Nageotte, J. (1930). *C.R. Soc. Biol., Paris*, **104**, 156.
 Nageotte, J. (1933). *C.R. Soc. Biol., Paris*, **113**, 841.
 Nageotte, J. & Guyon, L. (1933). *C.R. Soc. Biol., Paris*, **113**, 1398.
 Nageotte, J. & Guyon, L. (1934). *C.R. Ass. Anat.* **29**, 408.
 Neuberger, A., Perrone, J. C. & Slack, H. G. B. (1951). *Biochem. J.* **49**, 199.
 Neuberger, A. & Slack, H. G. B. (1953). *Biochem. J.* **53**, 47.
 Neuman, R. E. & Logan, M. A. (1950). *J. biol. Chem.* **184**, 299.
 Orekhovich, K. D. (1950). *C.R. Acad. Sci. U.R.S.S.* **71**, 521.
 Orekhovich, V. N. (1952). *2nd Int. Congr. Biochem. Communications*, p. 106.
 Orekhovich, V. N., Tustanovskii, A. A., Orekhovich, K. D. & Plotnikova, N. E. (1948). *Biochemistry, Leningr.*, **13**, 55.
 Perrone, J. C. (1951). *Nature, Lond.*, **167**, 513.
 Plotnikova, N. E. (1947). *C.R. Acad. Sci. U.R.S.S.* **58**, 1715.
 Robertson, W. van B. (1952). *J. biol. Chem.* **197**, 495.
 Schmitt, F. O., Hall, C. E. & Jakus, M. A. (1942). *J. cell. comp. Physiol.* **20**, 11.
 Sørensen, S. P. L. (1912). *Ergebn. Physiol.* **12**, 393.
 Tustanovskii, A. A. (1947). *Biochemistry, Leningr.*, **12**, 285.
 Udenfriend, S. & Cooper, J. R. (1952). *J. biol. Chem.* **196**, 227.
 Ussing, H. H. (1941). *Acta physiol. scand.* **2**, 209.
 Wyckoff, R. W. G. & Corey, R. B. (1936). *Proc. Soc. exp. Biol., N.Y.*, **34**, 285.
 Zachariades, P. A. (1900). *C.R. Soc. Biol., Paris*, **52**, 182.

Content of Vitamin A₂ in some Nile Fishes

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Heilbron, Gillam & Morton (1931) observed that in the antimony trichloride colour test, an absorption band at 693 m μ . accompanied the 617 m μ . band in many fish-liver oils. For several years, little progress was made in characterizing the new chromogen. It accompanied vitamin A in the richest concentrates and no separation could be effected either by chromatography or molecular distillation. Edisbury, Morton & Simpkins (1937) found relatively large quantities of the 693 m μ . chromogen in the eyes of gold-fish and the livers of trout. They therefore suggested that the 693 m μ . chromogen should be designated vitamin A₂, because its distribution suggested that it could replace vitamin A, particularly in fresh-water fish.

Morton, Salah & Stubbs (1946) prepared vitamin A₂ free from vitamin A₁, and later Morton, Salah & Stubbs (1947) suggested that it was dehydrovitamin A₁, with the extra double bond in the β -ionone ring.

From the retinas of marine fishes, Wald (1935) obtained rhodopsin from which, after bleaching, retinene₁ could be extracted by light petroleum. An analogous pigment, porphyropsin, was obtained from the eyes of fresh-water fishes (Wald, 1936); from this retinene₂ could be obtained. Morton & Goodwin (1944) proved that retinene₁ is vitamin-A₁ aldehyde and retinene₂ was later shown to be vitamin-A₂ aldehyde (Morton *et al.* 1947).

Wald (1937) has made the interesting observation that, in general, the eye tissues of fresh-water fishes contain vitamin A₂, while those of marine fishes contain vitamin A₁. Fishes which migrate from the one environment to the other contain both vitamins. It is with these facts in mind that Nile fishes have been examined. The amounts of both vitamin A₁ and A₂ in such fishes have not hitherto been determined. The results may help in accounting for distribution of vitamins A₁ and A₂ in nature.

EXPERIMENTAL

Materials. The fishes reached the laboratory within a few hours of being caught and were immediately dissected. The livers were removed and wet weights recorded.

Extraction of oils. The oils were prepared by grinding the livers with silver sand and anhydrous Na₂SO₄ and extracting with light petroleum 3 or 4 times. The solvent was completely distilled off in an atmosphere of CO₂ and the residue was weighed. The light petroleum used was purified by refluxing for 30 min. with KMnO₄ and dilute H₂SO₄; washed with water, then NaOH and water, and then dried over anhydrous CaCl₂. It was then fractionated and the fraction boiling between 40 and 60° was used.

Alumina. Alumina for chromatography (British Drug Houses Ltd.) was weakened by the addition of 10% of its weight of water and used immediately. The following is the procedure which was found suitable for weakening the active alumina. Alumina (10 g.) is made into a paste with

light petroleum and 1 ml. of water is added dropwise while the paste is triturated in a mortar. The alumina is kept under light petroleum and used the same day. In this way, the alumina produced was found suitable for chromatography and had no detrimental effect on the vitamin A present.

Maleic anhydride. This was recrystallized from benzene and then dissolved in A.R. benzene to give a 10% (w/v) solution.

Antimony trichloride reagent. Anhydrous antimony trichloride (20%, w/v) was dissolved in chloroform previously dried over CaCl_2 .

Spectrophotometry. A Unicam Photoelectric Spectrophotometer was used for the ultraviolet examinations, and a Hilger-Nutting Visual Spectrophotometer to locate the position of the bands in the antimony trichloride colour test.

Method of analysis of vitamin A in oil. Two methods were applied.

(a) A solution of the oil in CHCl_3 was prepared at a known concentration, such that when 0.2–0.4 ml. was treated with 4 ml. of antimony trichloride reagent the absorption of the blue solution at 617 and 693 $\text{m}\mu$. could be measured with the Hilger-Nutting Visual Spectrophotometer. Vitamin concentrations were calculated on the basis that vitamin A_1 has an $E_{1\text{cm}}^{1\%}$ 617 $\text{m}\mu$. of 5000 and vitamin A_2 has an $E_{1\text{cm}}^{1\%}$ 693 $\text{m}\mu$. of 4000.

(b) After chromatography and suitable dilution, the absorption curve was measured in the Unicam Spectrophotometer. After correction for irrelevant absorption (Morton & Stubbs, 1946) had been made, the potency was calculated on the basis of $E_{1\text{cm}}^{1\%}$ 325 $\text{m}\mu$. = 1700 for vitamin A_1 and $E_{1\text{cm}}^{1\%}$ 348 $\text{m}\mu$. = 1400 for vitamin A_2 . The following values were used in correcting curves corresponding with a predominance of vitamin A_2 :

$$E_{\text{max.}} = 348\text{--}350 \text{ m}\mu., E_{330 \text{ m}\mu.} = E_{368 \text{ m}\mu.}$$

and

$$E_{348 \text{ m}\mu.}/E_{330 \text{ m}\mu.} = 1.18.$$

These figures were obtained from a vitamin- A_2 curve on material seven-times chromatographed on 10% watered alumina.

Method of determining neovitamin A. The method is essentially that of Meunier & Jouanneteau (1948) which depends on the much more rapid formation of an adduct of

maleic anhydride with *all-trans*-vitamin A than with *neo*-vitamin A.

A weighed portion of the oil was dissolved in benzene. Suitable dilution gave a solution in benzene containing 200–300 i.u./ml. 5 ml. of this solution were pipetted into a flask and 5 ml. of maleic anhydride reagent added. After being shaken, the mixture was kept in the dark at 15° for 16 hr. Exactly 1 ml. was diluted for use in the antimony trichloride colour test. A measured volume of the untreated solution was tested in the same way. The colour was much weakened in the solution treated with maleic anhydride. The percentage of vitamin A existing in the *neo* form is given by the formula $\frac{R - R_1}{R_2 - R_1}$ where $R = \%$ recovery of vitamin A in the sample; $R_1 =$ that of vitamin A using a pure *all-trans*-vitamin A ester; $R_2 =$ that of *neovitamin-A* ester in a sample of ester free from *all-trans*-vitamin-A ester, the criterion of recovery being the appearance of the 620 $\text{m}\mu$. maximum in the colour test.

The values of R_1 and R_2 given by Meunier & Jouanneteau are 2 and 90, respectively. These same figures were also used for vitamin A_2 ; in the absence of direct calibration of *neovitamin A*₂, it has been assumed that *neovitamin A*₁ and *neovitamin A*₂ behave almost identically.

RESULTS AND DISCUSSION

The results of the determination of vitamin A_1 and A_2 and *neovitamin A*₂ in a number of Nile fish-liver oils are given in Table 1. This shows the vitamin-A content in the livers of sixteen species of Nile fishes. The survey has been limited to these species because they are the common varieties in Egypt and those which are consumed in preference to others by Egyptians.

The results show that Nile fishes, with the exception of *Anguilla aegyptica* and *Tilapia nilotica*, contain variable amounts of vitamin A_2 per g. liver oil, which amounts are, however, characteristic of the species. It was noticed that several species had a high vitamin- A_2 content, although the amount

Table 1. Vitamin-A content of liver oils of Nile fishes

Species*	Egyptian name	Weight in g. of fish	Oil in liver (%)	Vitamin A_1 ($\mu\text{g./g. oil}$)	Vitamin A_2 ($\mu\text{g./g. oil}$)	Neovitamin A_2 (% approx.)
<i>Tilapia nilotica</i> L.	Bolti	75–450	4–13	320–600	—	—
<i>Anguilla aegyptica</i> Shaw	Hannash	90–520	2–3	4000	—	—
<i>Barbus bynni</i> Forsk.	Bynni	220–1260	2.5–8	—	1430	17.3
<i>Labeo coubie</i> Rüpp.	—	220–660	2–6	—	840	—
<i>Labeo niloticus</i> Forsk.	Lebees	122–425	1–5	—	2600	—
<i>Mormyrus kannume</i> Forsk.	—	72–520	1.4–4.2	—	10 000	17.5
<i>Lates niloticus</i> L.	Homar	145–250	1–4	—	3000	20.2
<i>Chrysichthys auratus</i> Geoff.	Zammar	45–50	2.2–4.5	—	1230	21.5
<i>Clarias lazera</i> C. & V.	Karmout	95–440	1.5–9.4	—	3250	—
<i>Malopterurus electricus</i> Gn.	Raad	500–2000	1.6–3.4	—	13 000	—
<i>Synodontis schall</i> Bl. Schn.	Annuma	122–240	1.3–4.9	—	2500	—
<i>Schilbe mystus</i> L.	Shelba	65–142	2–5	—	70 000	—
<i>Bagrus bayad</i> Forsk.	Bayad	72–640	0.3–1.6	—	30 000	16.2
<i>Hydrocyon forskalli</i> Cuv.	Kelb-el-Bahr	440–1870	1.5–6	—	40 000	—
<i>Alestes dentex</i> L.	Rhy	55–525	2–15	—	4000	22.5

* See Boulanger (1907).

increased with the size of the fish. The presence of vitamin A₁ in *Anguilla aegyptica* and *Tilapia nilotica* is explained on the basis that the first is a migratory fish and the second lives mainly in the Delta region. It is interesting to notice that *Tilapia*

Fig. 1. Absorption spectrum of the liver extract in light petroleum from *Tilapia nilotica*. Maxima at 330 and 285, $E_{1\text{ cm}}^{1\%}$ 0.36 and 0.22.

Fig. 2. Absorption spectrum of liver extract in light petroleum from *Hydrocyanon forskali*. Maxima at 345 and 285, $E_{1\text{ cm}}^{1\%}$ 0.53 and 0.26.

Fig. 3. Light petroleum extracts of stomach and intestine of *Clarias lazera*. - - -, stomach; —, intestine.

is the fish most frequently appearing in the graphic and plastic representations of the ancient Egyptians.

Several samples of the various species were examined in the Beckman Spectrophotometer. Only *T. nilotica* and *Anguilla aegyptica* oils had a maximum at 330 mμ., indicating the presence of vitamin A₁. But the 285 mμ. band which is characteristic of vitamin A₂ was invariably present as shown in Fig. 1. The other species gave oils with a maximum at 245 mμ., together with another peak at 285 mμ. There was not a single exception. The oils of *Hydrocyanon forskali* and *Bagrus bayad* are exceptionally rich. Fig. 2 shows the absorption curve of *Hydrocyanon forskali*.

It was found that almost all the vitamin A₁ and A₂ occurred in the liver in the ester form. The vitamin-A₂ alcohol obtained after saponification gave an absorption spectrum in striking agreement with that of vitamin A₂ alcohol prepared by Ponndorf reduction of retinene₂.

The stomach and intestinal contents of *Clarias lazera* and *Lates niloticus* were examined spectroscopically and found to contain a mixture of carotenoids, maxima being recorded at 420, 440 and 470 mμ., as shown in Fig. 3 for *Clarias lazera*.

SUMMARY

1. The vitamin-A₁ and -A₂ contents of the livers of a number of species of Nile fishes have been determined.
2. The presence of neovitamin A₂ has been demonstrated.
3. Appreciable amounts of carotenoids did not occur in the livers of fishes, but carotenoids were present in the stomach contents of two species studied.

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REFERENCES

- Boulanger, G. A. (1907). *Fishes of the Nile*. London: Hugh Rees Ltd.
- Edisbury, J. R., Morton, R. A. & Simpkins, G. W. (1937). *Nature, Lond.*, **140**, 234.
- Heilbron, I. H., Gillam, A. E. & Morton, R. A. (1931). *Biochem. J.* **25**, 132.
- Meunier, P. & Jouanneteau, J. (1948). *Bull. Soc. Chim. biol., Paris*, **30**, 260.
- Morton, R. A. & Goodwin, T. W. (1944). *Nature, Lond.*, **153**, 405.
- Morton, R. A., Salah, M. K. & Stubbs, A. L. (1946). *Biochem. J.* **40**, lix.
- Morton, R. A., Salah, M. K. & Stubbs, A. L. (1947). *Nature, Lond.*, **159**, 744.
- Morton, R. A. & Stubbs, A. L. (1946). *Analyst*, **71**, 348.
- Wald, G. (1935). *J. gen. Physiol.* **19**, 351.
- Wald, G. (1936). *J. gen. Physiol.* **22**, 391, 775.
- Wald, G. (1937). *Nature, Lond.*, **139**, 1937.