fact that Munro's experiments were carried out under less satisfactory conditions; he himself states that more than half the tadpoles died during the first week, and that from the original 250 eggs only 10 survived to metamorphosis. Our own animals were well fed on a high-protein diet and had a high survival rate. Munro, on the other hand, does not state that his animals were fed but only that they were kept in water 'to which had been added an infusion of raffia, mud and weed obtained from a pond supporting a large frog population'.

According to our findings the changes during metamorphosis are similar to those found in other amphibians for which the ratio of ammonia to urea during development has been investigated. These are *Rana temporaria*, *Bufo bufo bufo*, *Triturus vulgaris*, *cristatus* and *Axolotl* (Munro, 1939, 1953). The only difference between *Xenopus* and these other species is that whereas *Xenopus* returns to a predominant excretion of ammonia after displaying a tendency to ureotelism during its larval life, the other species become and remain ureotelic during adult life. It appears in fact that a progressive tendency towards ureotelism is a feature of the larval life of amphibia in general, but, whereas the completion of metamorphosis is commonly attended by the development of total ureotelism, *Xenopus* shows only a transient move towards ureotelism and reverts towards ammoniotelism again even before the forelimbs begin to emerge.

Taken together with the observations of Cragg (1953) and Munro (1939, 1953), our results suggest that if *Xenopus* were to live a terrestrial or semi-terrestrial instead of an aquatic life, it would be capable of meeting the problems of nitrogen disposal in the manner usual among amphibia, i.e. by

ureotelism. Indeed, it seems likely that its survival during the dry season depends upon precisely this device. Certainly the dependence of *Xenopus* on an aquatic habitat is not to be attributed to failure to develop the machinery necessary for ureogenesis.

SUMMARY

1. The excretion of nitrogen in the form of ammonia and urea by *Xenopus* tadpoles has been studied up to the end of metamorphosis.

2. There is a marked increase in the relative proportion of urea nitrogen in the early stages of metamorphosis followed by a fall as metamorphosis proceeds.

3. The total nitrogen excreted/gram body weight/24 hr. shows a sharp increase in the late stages of metamorphosis.

Our thanks are due to Dr D. R. Newth, who kindly provided the tadpoles used in this work, to Dr Mavis Cragg for permission to quote from her unpublished results, and to Dr W. H. Hampton, who first drew our attention to the fact that *Xenopus* tadpoles excrete urea.

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The Branched-Chain Fatty Acids of Ox Fat

3. THE ISOLATION OF 14-METHYLPENTADECANOIC ACID (ISOPALMITIC ACID) FROM OX PERINEPHRIC FAT*

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Ox perinephric fat has been shown to contain trace quantities of the branched-chain fatty acids (+)-14-methylhexadecanoic acid (Hansen, Shorland & Cooke, 1952) and 15-methylhexadecanoic acid (Hansen, Shorland & Cooke, 1955b); in this paper is reported the occurrence of the C_{18} iso-acid 14-methylpentadecanoic acid.

* Part 2: Hansen *et al.* (1955b).

EXPERIMENTAL

As reported earlier (Hansen *et al.* 1955b) fractionation of the 'liquid' methyl esters obtained from hydrogenated ox perinephric fat yielded 22 fractions, the following four of which pertain to this paper: J/21, L12, wt. 38.06 g., saponification equiv. 272.1, m.p. 22.8–23.8°; J/21, L13, wt. 41.59 g., saponification equiv. 272.3, m.p. 23.9–25.9°; J/21, L14, wt. 41.72 g., saponification equiv. 273.1, m.p.

25.8–26.5°; J/21, L15, wt. 40.78 g., saponification equiv. 275.2, m.p. 17.2–19.2°.

These were combined (denoted U10), freed from unsaponifiable matter and refractionated as methyl esters in column C (Shorland, 1952) to give 18 fractions and a residue. As the first 14 fractions (U10L1 to U10L14) all possessed saponification equiv. approximating to 270 and m.p.'s ranging between 22.0° and 28.5°, they were combined (129 g.) and as methyl esters crystallized 4 times from 40 vol. of acetone at –45°. The soluble fractions which were separated from the insoluble fractions after each crystallization were bulked (denoted U31, wt. 25.3 g.) and refractionated in column E (Shorland, 1952) to yield 13 fractions (U31L1 to U31L13) and a residue (U31LR) as shown in Table 1.

Table 1. *Fractionation of U31 methyl esters (25.3 g.)*

Fraction	Wt. (g.)	Saponification equiv.	M.p.	
U31L1	1.50	259.3	1.7–5.5°	
U31L2	3.35	260.1	6.0–7.0	
U31L3	2.97	266.1	12.5–13.2	U43
U31L4	2.19	267.7	14.0–14.5	
U31L5	1.77	268.6	12.8–14.0	
U31L6	2.27	269.5	13.0–13.5	
U31L7	0.35	269.0	12.2–13.5	
U31L8	1.55	269.8	10.0–13.0	
U31L9	1.51	269.4	10.8–11.9	
U31L10	1.46	270.7	6.0–8.2	
U31L11	1.57	276.1	4.7–7.3	
U31L12	0.92	280.2	1.0–5.0	
U31L13	1.47	284.4	1.0–3.3	
U31LR	1.98	378.1	—	

Fractions U31L3 to U31L9 inclusive were combined as acids (denoted U43, wt. 11.89 g.) and submitted to 20 crystallizations, 4 times from 40 vol. of light petroleum (b.p. 50–60°) at –40°, once from 40 vol. of light petroleum (b.p. 50–60°) at –70°, 3 times from 40 vol. of acetone at –40°, 4 times from 20 vol. of ether at –70°, once from 100 vol. of ether at –25°, once from 40 vol. of methanol at –40°, and 6 times from 20 vol. of ether at –40°. The final separation resulted in a soluble fraction U43S12LS6L (A), wt. 0.25 g., m.p. 58.0–59.6°, and an insoluble fraction U43S12LS6S (B), wt. 5.50 g., m.p. 62.0–62.6°. (In all crystallizations the soluble fraction was removed and the insoluble fraction recrystallized except where 100 vol. of ether at –25° was employed, and there the resulting soluble fraction was recrystallized.) Fatty acid fraction B had m.p. 62.0–62.6°. Arosenius, Stållberg, Stenhagen & Tägtström-Eketorp (1949) record m.p. 61.5–61.6°, Fordyce & Johnson (1933) 61.8–62.4°, Weitkamp (1945) 62.4° and Milburn & Truter (1954) 61.4–62.0°. The mixed m.p. with an equal wt. of 14-methylpentadecanoic acid (recorded m.p. 62.4°, our value 62.3–62.4°) supplied by Weitkamp (1945) was 62.2–62.6°. Mixed with an equal wt. of *n*-hexadecanoic acid (m.p. 62.9–63.3°) it gave m.p. 49.7–50.6° (cf. Weitkamp, 1945). It had n_D^{20} 1.4293, X-ray long spacing 30.24 ± 0.5 Å (Arosenius *et al.* 1949 record 30.4 Å), iodine value 0.0, and *C*-methyl value 6.4%, equiv. to 1.1 mol. of acetic acid (cf. Ginger, 1944). (Found: C, 75.0; H, 12.5%; saponification equiv., 256.3. Calc. for $C_{16}H_{32}O_2$: C, 75.0; H, 12.6%; saponification equiv., 256.4.) The methyl ester had m.p. 16.9–17.7°; n_D^{20} 1.4402.

In the X-ray work manganese-filtered iron $K\alpha$ radiation was used and the measurements were made with a Philips Geiger-counter X-ray spectrometer. The sample was melted on a glass slide and cooled.

All melting points were determined in closed capillaries and are uncorrected.

Both the combustion analysis and the *C*-methyl determination were made by Weiler and Strauss, Oxford.

DISCUSSION

The chemical and physical properties of fraction B identify it as the C_{16} *iso*-acid 14-methylpentadecanoic acid.

*iso*Palmitic acid has been synthesized (Fordyce & Johnson, 1933; Arosenius *et al.* 1949; Milburn & Truter, 1954), and has been identified in wool wax (Weitkamp, 1945). It has not, however, been formerly isolated from a natural fat. Indeed, only one other even-numbered *iso*-acid, 12-methyltridecanoic acid (*isomyristic acid*), has been located in a natural fat (Hansen, Shorland & Cooke, 1954*b*). Although the complete series of even-numbered *iso*-acids from C_{10} to C_{28} inclusive has been shown to be present in wool grease (Weitkamp, 1945), no odd-numbered *iso*-acids have been reported there as they have in butterfat (Hansen & Shorland, 1951; Hansen, Shorland & Cooke, 1954*a*; Shorland, Gerson & Hansen, 1955; Hansen, Shorland & Cooke, 1955*a*), mutton fat (Hansen, Shorland & Cooke, 1953) and ox fat (Hansen *et al.* 1955*b*).

The occurrence of both odd- and even-numbered *iso*-acids in natural fats could be explained by deamination in the rumen of the appropriate amino acids, namely, valine and leucine, to give *isobutyric* and *isovaleric* acids respectively (cf. El-Shazly, 1952). Successive condensation of these acids with acetate could give the odd and even series of *iso*-acids found in natural fats.

The present investigation suggests that 14-methylpentadecanoic acid occurs in hydrogenated ox perinephric fat to the extent of approximately 0.1% of the weight of the total fatty acids.

SUMMARY

14-Methylpentadecanoic (*isopalmitic*) acid is shown to be present in ox perinephric fat and is estimated to represent 0.1% of the weight of the total fatty acids.

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The Annelid Phosphagens

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After the work of Eggleton & Eggleton (1929) and of Meyerhof (1928) it was generally assumed that the creatine phosphate characteristic of vertebrate muscle is always replaced by arginine phosphate in the muscle of invertebrates. A broad comparative study of invertebrate animals by Needham, Needham, Baldwin & Yudkin (1932) did not reveal the occurrence of any phosphagen other than arginine phosphate, with certain notable exceptions among the echinoderms. Greenwald (1946), however, had shown that the reproductive organs of certain marine annelids contain a material that is strongly chromogenic in the Jaffe reaction and thus might be creatine. Baldwin & Yudkin (1950) described the presence of an arginine-like phosphagen in annelid and geophyean worms and showed that a second phosphagen, possibly creatine phosphate, is present in certain annelids. The work reported here, carried out on annelids at Roscoff in 1951, demonstrates that the creatine-like substance described by Baldwin & Yudkin (1950) in annelids is in fact creatine phosphate and extends the examination to a number of annelid species not previously studied.

By the use of paper chromatography during the period 1951-4, Roche and his co-workers have shown the presence of new guanidine derivatives in annelids. Thoai, Roche, Robin & Thiem (1953a) have isolated taurocyamine and glycocyamine from annelids and glycocyamine from the nemertine *Lineus gesserensis* (Thoai, Roche, Robin & Thiem (1953b)). They postulated that these bases occur as phosphates and constitute the arginine-like phosphagen described by Baldwin & Yudkin (1950). Some of this work has been confirmed.

METHODS

Preparation of tissue extracts. Freshly collected animals were cooled in sea water to 0° before use. The larger species were gutted before being extracted; the smaller were extracted whole. The chilled material was ground with sand and extracted in an ice-cold mortar with ice-cold 8% (w/v) trichloroacetic acid, usually in the proportion of 5 ml./g. of tissue. The extract was centrifuged at about 600 g at 0°. The supernatant was neutralized (pink to phenolphthalein), and the $\text{Ca}(\text{OH})_2$ - CaCl_2 reagent of Fiske & Subbarow (1929) was added to precipitate the inorganic phosphate. After standing 10 min. in ice the tubes were centrifuged. Phosphagens were estimated in the supernatant by the method described by Baldwin & Yudkin (1950).

For chromatographic analysis, the neutralized extract prepared as described above was placed on Whatman no. 1 paper. In some cases the phosphagens were first concentrated by precipitation at 0° with 3 vol. of ice-cold acetone and purified by dissolving in the minimum quantity of water and reprecipitating with acetone.

The solvent suggested by Hanes & Isherwood (1949), *n*-propanol-ammonia (sp.gr. 0.880)-water (60:30:10), was used and was allowed to flow down the paper. Up to 0.05 ml. of the extract was put on the paper, the diameter of the spot always being kept below 0.7 cm. The chromatograms were run at room temperature (22°) usually for 6 hr.; during this time the solvent front moved about 25 cm. An alternative solvent system of Thoai & Robin (1954) [isopentanol-pyridine-water (40:80:70)] was also used.

Creatine phosphate. This was prepared by the method of Ennor & Stocken (1948).

Taurocyamine phosphate and glycocyamine phosphate were prepared enzymically as described by Hobson (1955).

RESULTS

In 1951 species hitherto unexamined were analysed for the creatine-like and arginine-like phosphagens by a hydrolysis method identical in all essentials