SUMMARY

Tadpoles and newly-metamorphosed specimens of *Triturus vulgaris* L. in a pond in the Rock Garden of the Botanical Garden of Gothenburg in western Sweden were studied during 1970 and 1971. In both years adults were found in the pond during two periods, one in spring and one in early summer. In 1970 animals spawned only during the first period. In 1971 the two spawning periods were each followed by periods of egg hatching and metamorphosis. The pond accommodated two fullgrown tadpoles per m^2 (= 2.25 per female in 1970 and 1.64 in 1971), and of these more than two thirds metamorphosed. Tadpoles grew somewhat faster in 1971, possibly owing to higher water temperature. A few *T. cristatus* Laur. were observed in the pond, but their reproduction here was negligible.

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FIGURE 1.
The experimental pond and relevant trapping equipment.

FIGURE 2.
The trap used to capture newly-metamorphosed newts.

FIGURE 3.
Number of adult *Triturus vulgaris* in the pond in 1970.

FIGURE 4.
Number of adult *Triturus vulgaris* in the pond in 1971.

FIGURE 5.
Number of tadpoles of *Triturus vulgaris* in the pond.

FIGURE 6.
Number of newly-metamorphosed *Triturus vulgaris* in 1970.

FIGURE 7.
Number of newly-metamorphosed *Triturus vulgaris* in 1971.

FIGURE 8.
Water temperature of the pond.

	Number of specimens caught in		Average number of times each specimen was caught in		Number of specimens caught in both years
	1970	1971	1970	1971	
Males	5	13	1.4	1.7	—
Females	8	11	1.6	1.8	2

Table 1

A COMPARISON OF LIPIDS FROM THE FAT BODY AND TAIL OF THE COMMON LIZARD, *LACERTA VIVIPARA*

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INTRODUCTION

It has long been known that the abdominal fat bodies of lizards, like those of most reptiles, are major storage reserves, and that they vary in size from season to season. More recently it has been shown that fat stored in the tail of the Common lizard *Lacerta vivipara* Jacquin, fluctuates in a similar way, and that the quantities of stored material at the two sites are approximately equal (Avery, 1970). In this paper we report an analysis of lipids from the two sites, carried out in order to determine if there were any differences in composition between them.

MATERIAL AND METHODS

Six adult male lizards were captured at Priddy in Somerset between 16 March and 21 March, 1972, i.e. during the period immediately after hibernation. The abdominal fat bodies and the tail fat deposits were removed by dissection and stored in iso-propyl alcohol with 0.0005% butylated hydroxytoluene as antioxidant. The material was pooled for analysis.

Lipids were extracted in isopropanol/chloroform mixtures (Thomas & Stobart, 1970) and fractionated into polar and neutral components (Stobart & Pinfield, 1970) which were estimated gravimetrically. Methyl esters of the neutral lipid fatty acids were prepared by transmethylation and separated by gas chromatography. Glycerides were separated into saturation groups by thin layer chromatography using 0.25 mm Kieselgel plates which were impregnated with 5% $AgNO_3$ and developed in a CCl_4 — $CHCl_3$ — glacial acetic acid—ethanol mixture. Diglycerides were seperated from triglycerides by TLC using Kieselgel plates and a light petroleum 40-60° — diethyl ether — glacial acetic acid mixture (Shewry, Pinfield & Stobart, 1972). After development, TLC plates were sprayed with 50% sulphuric acid and charred for 20 min at 220°C. Quantitative measurements were made by densitometer tracings of the charred areas using a Joyce-Loebl chromoscan. Polar lipids were fractionated into individual phospholipids by TLC with acid and basic solvent mixtures (Nichols, 1964). Free sterols were precipitated as the digitonides which were redissolved in glacial acetic acid and estimated by the Lieberman-Burchard reaction (Moore & Baumann, 1952). Amounts of total free sterols were calculated from a standard cholesterol calibration curve.

RESULTS

COMPONENT ACIDS

95.5% of the lipids from both the fat body and the tail fractionated into neutral components, and 4% into polar components. The polar lipids were predominantly phosphatidyl ethanolamine and phosphatidyl choline.

The major neutral lipid fatty acids which were identified by gas chromatography according to their positions relative to a palmitate standard, are shown in Table 1. The most abundant corresponded to oleic acid, and comprised slightly more than half of the total neutral fatty acid in both tissues. Oleic acid is an unsaturated fatty acid with eighteen carbon atoms and a single double bond in the molecule; it has the structural formula $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ and is usually represented as $C_{18:1}$ (see Table

1). There were also relatively large quantities of palmitic ($C_{16\ 0}$), linoleic ($C_{18\ 2}$) and linolenic ($C_{18\ 3}$) acids. (There is an excellent account of lipid classification and its biological significance, written for the non-biochemist, in an article on "Fish Nutrition" by C. B. Cowey & J. R. Sargent in *Advances in Marine Biology* vol. 10, 1972). Differences in the percentages of individual acids in the fat body and the tail were small and are almost certainly not significant, although a possible exception is that of linolenic acid, which comprised 10.46% of the neutral fatty acids in the fat body, but only 6.95% of those in the tail.

Component glycerides

Chromatographic separation of glycerides by argentation TLC is based on the degree of unsaturation in the molecule; consequently each component does not usually represent an individual glyceride, but a number of glycerides having the same degree of unsaturation.

Eleven saturation groups were found in both tissues; the percentages of each are shown in Table 2. The differences are almost certainly not significant, with the possible exceptions of those in groups 8 and 9. Triglycerides comprised 88% of the fat body neutral lipids and 91% of the tail neutral lipids (Table 3), whilst diglycerides comprised 9% and 4% respectively. The larger amounts of glyceride in groups 8 and 9 in the fat body neutral lipid fraction were possibly related to the high amounts of linolenic acid ($C_{18\ 3}$) and diglycerides in this fraction.

Sterols estimated as cholesterol comprised less than 1% of the total neutral lipid in both tissues.

DISCUSSION

There have been very few studies of reptile lipids, and none of those of the tail. Nearly all of the reptile lipids which have been analysed have a high proportion of unsaturated C_{18} acids, with oleic acid predominating (Hilditch & Williams, 1964; Grenot, 1968). Zain & Zain-ul-Abedin (1967) have noted that the fat bodies of the desert lizard *Uromastix hardwickii* contain 90% of esterified fatty acids, and are therefore more equivalent to white adipose tissue than to the brown fat which is associated with mammalian hibernation, since brown fat contains relatively more glycogen, phospholipid and cholesterol, and relatively less neutral lipid. Our resuts are consistent with both of these findings.

Although the abdominal fat bodies and the fat deposits around the tail vertebrae are quite distinct sites, there are no major differences in their composition. Since only one analysis of each tissue has been made, we are not able to determine whether the small differences in the percentages of linolenic acid and diglycerides are significant, but the related differences in the percentages of glyceride groups provide circumstantial evidence that they are. It is interesting in this context that amounts of fat body linolenic acid fluctuated more widely between four North African lizard species than those of six other major fatty acids which were measured (Grenot, 1968).

Since the composition of the deposits in the two tissues is essentially similar, it is perhaps not surprising that their lipids are utilised during the course of hibernation to a similar extent (Avery, 1970). A more detailed account of lipid storage and release in lizards of different age, sex and weight at different times of the year, will be published elsewhere.

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Identity	$^{c}12:0$	$^{c}14:0$	$^{c}16:0$	$^{c}16:1$	$^{c}18:0$	$^{c}18:1$	$^{c}18:2$	$^{c}18:3$	others
Position relative to palmitate	0.31	0.56	1.00	1.24	1.78	2.07	2.80	3.91	4.0
Percentage in fat body	0.91	1.63	13.08	5.85	4.13	51.40	11.88	10.43	2.0
Percentage in tail	1.23	2.28	14.85	6.41	3.91	52.13	11.64	6.95	2.0

Table 1. Neutral fatty acid content of the fat body and the tail. Results are expressed as percentages of the total neutral lipid fatty acid.

Glyceride group number	1	2	3	4	5	6	7	8	9	10	11
Relative front (Rf)	0.89	0.83	0.76	0.65	0.57	0.47	0.37	0.31	0.25	0.19	0.15
Percentage in fat body	3.49	14.90	23.57	11.77	10.12	12.42	5.33	5.65	6.76	3.50	2.49
Percentage in tail	2.42	14.54	27.22	13.09	12.19	12.92	5.67	3.13	3.90	2.70	2.22

Table 2. Glycerides of the fat body and tail. Glyceride group numbers refer to the relative positions of the glycerides on $AgNO_3$-impregnated TLC plates; the results are expressed as percentages of total glyceride.

Spot number	1	2	3
Identity	diglyceride	unknown	triglyceride
Relative front (Rf)	0.30	0.35	0.74
Percentage in fat body	9.10	3.09	87.81
Percentage in tail	4.07	3.51	91.42

Table 3. Total diglyceride and triglyceride in the fat body and tail, expressed as percentages of the total glyceride.

INFLUENCE OF PHOTOPERIOD AND LIGHT INTENSITY ON LIZARD VOLUNTARY TEMPERATURES

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Regal (1967) and Gehrmann (1971) have shown that reptiles kept in temperature gradient chambers exhibit a 24-hour rhythm in their voluntary temperatures (body temperatures associated with normal activity), which is synchronised with a light dark cycle. Although this phenomenon has been considered in detail and although several explanations proffered, the parameter of light intensity has not been discussed in connection with reptile voluntary temperatures.