Catecholamine-containing nerve cell bodies and varicose nerve fibers have been found previously in human fetal brain (*6*). The results reported here demonstrate the presence of catecholamine-containing nerve terminals in the cerebral and cerebellar cortices of children and adults. We found no principal qualitative differences in appearance between the catecholamine nerve fibers in rat and those of man with the smear technique; this suggests that the knowledge about the fluorescence histochemistry of catecholamine neuron systems in laboratory animals might apply to the cerebral cortices of man.

The specificity and high sensitivity of the Falck-Hillarp fluorescence method have been discussed (*7*), and a change in fluorescence intensity represents a change in the NA content of adrenergic nerves (*8*). With the smear technique, optimal conditions for detection of fluorescent nerves in sparsely innervated brain areas are obtained. The smear technique has been used to quantitate the effect of minor tranquilizers on NA turnover in the rat cerebral and cerebellar cortices (*9*), and there seems to be a linear relation between the endogenous amount of NA in the rat cerebral cortex and the fluorescence intensity estimations obtained with the smear technique on coded slides (*10*). Thus, this technique should also permit quantification of nerve endings and fluorescence intensities in various clinical conditions in man.

No major regional differences in the distribution of NA nerve terminals in the rat cerebral cortex have been reported (*11*). In our study of the human brain, fluorescent terminals were found in the cortices of the frontal, temporal, and parietal lobes as well as in the parieto-occipital area (Table 1). Therefore, the lack of fluorescent terminals in one case might reflect the serious condition of this patient.

In the rat, the catecholamine nerve terminals in the cerebral and cerebellar cortices are of the NA type (*12*), and they have a common origin in the NA nerve cell bodies of the locus ceruleus (*13*). There is thus reason to believe that the nerves with green fluorescence in the human cortices are of the NA type.

The results of the initial experiments suggest that fluorescence histochemistry of brain smears may offer new possibilities to the study of central monoamine neurons in man. Apart from obtaining the necessary background of anatomy, we found with this technique that disturbances in nerve terminal frequency as well as in monoamine content may be directly related to various clinical conditions.

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14. Supported by the Swedish Medical Research Council grants B70-14X-2877-02 to B.N., B71-14X-3185-01 and B71-14X-3185-02 to L.O., and B72-14X-3574-01 to U.U., and by Magnus Bergvalls Stiftelse. We thank M. Eliasson for technical assistance.

20 December 1971; revised 7 March 1972

Triacylglycerols Characteristic of Porpoise Acoustic Tissues: Molecular Structures of Diisovaleroylglycerides

Abstract. *More than two-thirds of the triacylglycerols from the acoustic tissues of the porpoise* (Tursiops gilli) *consist of 2 moles of isovaleric acid for every 1 mole of long-chain acids. Cranial blubber, which has no distinct acoustic function, does not contain these unusual glycerides. The presence of large amounts of diisovaleroylisopentadecanoylglycerol suggests that this structure may be particularly important in sound transmission through lipid-protein matrices.*

Porpoise acoustic tissues, such as those from the melon and mandibular canal, are composed of unusual wax esters and triacylglycerols rich in isovaleric acid and long-chain iso acids (*1*, *2*). However, blubber taken from an area of the head in close proximity to the melon (cranial region) but having no distinct acoustic function contains primarily straight-chain unsaturated acids (*3*) characteristic of normal mammalian adipose tissue (*4*).

The unusual composition of the acids in acoustic tissues has evoked an interest in the molecular structure of the triacylglycerols. At present, data on cetacean acoustic tissues are limited to analyses of the carbon numbers of hydrogenated triacylglycerols from the beluga whale (*Delphinapterus leucas*) (*5*). For current interdisciplinary studies on bioacoustics (*6*), a better understanding of the structural and biochemical role of lipid in the acoustic tissues is of paramount importance. We report here the analyses of individual triacyl-

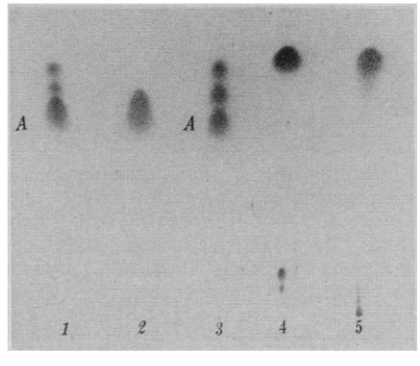

Fig. 1. Thin-layer chromatography of triacylglycerols on silica gel. Solvent: a mixture of hexane and diethyl ether (90 : 10, by volume); indicators: 0.5 percent solution of iodine in chloroform and starch solution. Fractions *A* do not stain as well as the more unsaturated upper fractions. Thus, visual estimation of relative amounts of the zones is misleading. *1*, Melon triacylglycerols from *Tursiops gilli*; *2*, diisovaleroylmyristoylglycerol; *3*, mandibular canal triacylglycerols from *Tursiops gilli*; *4*, trioleoylglycerol; and *5*, blubber triacylglycerols from *Tursiops gilli*.

glycerols from the melon and mandibular canal of the porpoise (*Tursiops gilli*) that are not present in blubber from the cranial region. The data suggest an important role for these compounds in the architecture of porpoise acoustic tissues.

The percentages of total lipid in the melon, mandibular canal, and blubber tissues of *T. gilli* were 70.0, 46.1, and 18.4 percent, respectively, of which triacylglycerols comprised 75 to 88 percent. The triacylglycerols were isolated and purified as previously described (*2, 3*). Thin-layer chromatography of the purified triacylglycerols from the melon and mandibular canal on activated layers of silica gel (*7*) resulted in the separation of three distinct fractions, whereas triacylglycerols from the blubber did not reveal any separation into subclasses (Fig. 1). The major fractions (*A*) from the melon ($R_F = 0.60$) and mandibular canal ($R_F = 0.58$) corresponded to marker diisovaleroylmyristoylglycerol ($R_F = 0.59$). The single zone of triacylglycerols from the blubber had an R_F value (0.72) similar to that of marker trioleoylglycerol ($R_F = 0.74$). Isolation, followed by gravimetric analysis (*8*), revealed that fractions *A* from the melon and mandibular canal comprised 78.8 and 67.4 percent of the total triacylglycerols, respectively. No evidence was found for triacylglycerols in the chromatoplate of blubber after elution of an area of silica gel corresponding to fraction *A*.

Detailed analyses are restricted to the major fractions (*A*) of the triacylglycerols, that are distinctly associated with porpoise acoustic tissues. Acyl moieties of triacylglycerols were analyzed as butyl esters by gas-liquid chromatography as described previously (*2*). Intact triacylglycerols were examined at 198°C by gas-liquid chromatography (Barber-Colman model 5000) on a glass column (67 by 0.32 cm) packed with 3 percent JXR on Chromosorb Q (100 to 120 mesh). Individual peaks were identified by the use of pure triacylglycerol standards (Applied Science Laboratories). The triacylglycerols were also analyzed at 162°C to detect the presence of triisovaleroylglycerol.

No evidence was found for the presence of triisovaleroylglycerol in either acoustic tissue. Moreover, analyses of acyl groups showed that the mole ratio of isovaleric acid to total long-chain acids (C_{12} to C_{18}) was 2:1 in triacylglycerols from both the melon and mandibular canal. Thus, the triacylglycerols were composed entirely of 2 moles of isovaleric acid and 1 mole of long-chain acids.

Table 1. Triacylglycerols containing 2 moles of isovaleric acid from the acoustic tissues of the porpoise (*Tursiops gilli*). Values are expressed in mole percent.

Chain length	Melon		Mandibular canal	
	Acids*, †	Diisovaleroyl-glycerides	Acids*, ‡	Diisovaleroyl-glycerides
12:0 iso	0.3	0.8§	0.2	0.2§
12:0	0.2		0.3	
13:0 iso	0.4	3.0§	0.3	1.5§
13:0	0.3		0.4	
14:0 iso	2.5	6.7	3.7	10.3
14:0	3.2	8.4	4.9	12.4
15:0 iso	16.8	49.0	12.5	35.2
16:0 iso	1.5	5.8	4.4	14.9
16:0	3.7	11.1	4.7	15.6
16:1	2.4	10.9	0.5	2.4
18:1	1.3	5.3	1.6	6.8

* Acids derived from triacylglycerols (fractions *A*, Fig. 1). † Contains 65.9 mole percent isovaleric acid. ‡ Contains 66.2 mole percent isovaleric acid. § Not separable.

The separation and characterization of the individual diisovaleroylglycerides were readily accomplished by gas-liquid chromatography because differences in molecular structures were associated only with long-chain acyl groups. Because isovaleric acid represents two-thirds of the total acids of the triacylglycerols and each glycerol moiety contains two isovaleroyl chains, the composition of the triacylglycerols could be calculated by multiplying the values for long-chain (C_{12} to C_{18}) acids by a factor of 3 (Table 1). Despite the differences in column conditions for the analyses of acids and intact triacylglycerols, a close correlation was obtained between both sets of values.

Under the conditions of gas-liquid chromatography, 1,2 and 1,3 isomers were not separable. In addition, attempts to determine the positional distribution of acyl chains was hampered by the strong resistance of these triacylglycerols to hydrolysis by porcine pancreatic lipase, as reported previously (*9*). The resistance to lipase hydrolysis may be related to the preponderance of branching which would be expected to sterically hinder the formation of the activated complex between the enzyme and the ester linkage (*10*). Further studies with other lipolytic enzymes may lead to suitable methods for the determination of isomeric structure in these unusual glycerides.

A significant finding is the fact that the large amount of isovaleric acid present in the acoustic tissues is not randomly distributed, but is almost exclusively associated with diisovaleroylglycerides. Moreover, diisovaleroylglycerides comprise more than two-thirds of the total triacylglycerols of the acoustic tissues, whereas the blubber triacylglycerols do not contain detectable amounts of these glycerides. The triacylglycerols of the melon and mandibular canal comprised 84.8 and 90.1 mole percent of the saturated structures, respectively. Moreover, glycerides containing branched-chain acids on each position of glycerol represent more than half of the total triacylglycerols in both acoustic tissues (Table 1). Considering the possibility that two isomeric triacylglycerols (1,2- and 1,3-diisovaleroyl derivatives) may exist, no more than 22 positional isomers can be deduced from the data in Table 1. Thus, the triacylglycerols distinctly associated with acoustic tissues are remarkably simple in comparison to highly complex mixtures of triacylglycerols present in most marine animals (*11*).

Diisovaleroylisopentadecanoylglycerol was the major component in both the mandibular canal (35.2 mole percent) and melon (49.0 mole percent) (Table 1). The occurrence of large amounts of this glyceride in the acoustic tissues suggests that this compound, in particular, may play an important role in the transmission of sound through lipid-protein matrices. A study of the acoustical properties (for example, compressibility and acoustical impedance) of diisovaleroylisopentadecanoylglycerol may provide further clues to the role of branched-chain triacylglycerols in the architecture of porpoise tissues involved in biosonar.

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7 January 1972; revised 31 March 1972 ■

Call Types of the Rana pipiens Complex in Illinois

Abstract. *The presence of western, eastern, and northern call types of the* Rana pipiens *complex in Illinois supports evidence from the western United States that this complex can no longer be regarded as one species.*

Littlejohn and Oldham (*1*) found populations of the *Rana pipiens* complex (leopard frogs) in the western United States that differed markedly in mating calls (*2*). Four species were recognized (designated western, eastern, northern, and southern call types). Other work indicated a fifth call type in Arizona and New Mexico (*3, 4*). Sympatric records in Texas, Arizona, and Colorado (*1, 3–5*) indicate that the call types are maintaining reproductive isolation. The significance of this research is twofold. First, it casts doubt on the validity of frequently cited work (*6*) that regarded *"R. pipiens"* as a single species with populations from different regions showing varying degrees of genetic incompatibility. Second, previous experimental studies that revealed variation in *"R. pipiens"* may be unreliable because experimental material may have included two or more species. This is particularly important because of the extensive use of *"R. pipiens"* in experimental research.

Species names were not applied to the call types because topotypic calls of formerly described members of the complex were lacking, and the status of the complex in other areas of North America was unknown (*1*). We report the first records of the northern, eastern, and western call types in Illinois.

Calls were recorded with a Stancil-Hoffman Minitape M9 tape recorder and Electro-Voice 644 microphone. Temperatures were taken with a Schultheis quick reading thermometer (*7*). Recordings were analyzed with a Kay model 6061A Sona-Graph (*8*). Frog calls were recorded in the spring of 1970 and of 1971 at the following localities: northern call type, 9.0 km north of Grand Detour, and 9.1 km west of Ottawa; western call type, 14.2 km north of Normal; eastern call type, 11.1 km south of Bath.

The call types in Illinois have calls (Table 1) similar to those of the corresponding call types reported previously (*9*). Pulse durations of the northern call type from Illinois, however, are shorter than those previously reported (*1*). The distinctness of the call types from Illinois is most clearly seen in Table 2, because temperature variation is minimal (17.2° to 18.2°C). Presumed western call type males were heard (but not recorded) in sympatry with the northern call type at the Ottawa locality, and with the eastern call type at Bath. No hybrids were encountered. Only one call type was heard at the other localities.

Call types can often be distinguished by morphology, but overlapping character variation (*1, 4*) makes positive identification dependent on call analysis. Dorsolateral folds are usually displaced in the western call type and continuous in the northern and eastern call types. Vestigial oviducts are present in males of the northern call type but absent in males of the western and eastern call types. The three call types recorded in Illinois agree with these diagnostic characters. Another call type from New Mexico and Arizona (*4*) is similar to the western call type in that it has displaced dorsolateral folds and lacks male oviducts. However, the two call types have distinctive calls (*1, 4*).

Most previous work on the call types was carried out in the western United States. The Illinois records thus represent range extensions of considerable magnitude. The Ottawa record for the

Table 1. Some call characteristics of three call types of the *Rana pipiens* complex from Illinois compared with calls from other localities reported by Littlejohn and Oldham (*1*). Mean values are given above ranges which are in parentheses.

Localities	Individuals (No.)	Temperature (°C)	Pulse rate (No./sec)	Duration	
				Call (sec)	Pulse (msec)
			Western call type		
Illinois	5	22.1	5.1	0.68	24.0
		(21.8–22.5)	(4.9–5.3)	(0.57–0.77)	(19.8–29.0)
Texas*	7	. . .	5.6	0.66	27.0
		(20–25)	(4.6–6.8)	(0.48–0.89)	(23–35)
			Eastern call type		
Illinois	2	21.7	12.0	0.46	42.1
		(21.5–21.8)	(11.8–12.3)	(0.39–0.53)	(41.9–42.3)
Texas*	7	. . .	14.8	0.41	39.4
		(20–25)	(14.3–15.3)	(0.31–0.52)	(33–50)
			Northern call type		
Illinois	1	15.9	14.2	2.86	7.1
Colorado*	4	. . .	13.7	3.75	17.8
		(12–16)	(12.9–14.6)	(3.30–4.73)	(16–20)

* Data from Littlejohn and Oldham (*1*).

Table 2. Some call characteristics of three call types of the *Rana pipiens* complex from Illinois recorded at 17.2° to 18.2°C. Mean values are given above ranges which are in parentheses.

Call type	Individuals (No.)	Temperature (°C)	Pulse rate (No./sec)	Duration	
				Call (sec)	Pulse (msec)
Western	2	18.2	3.6	0.69	36.4
		(18.2)	(3.6–3.7)	(0.59–0.78)	(34.4–38.3)
Eastern	4	17.7	9.0	0.88	70.4
		(17.2–18.2)	(8.5–9.3)	(0.74–1.03)	(67.3–73.3)
Northern	5	17.9	19.4	2.90	7.3
		(17.6–18.2)	(17.0–21.2)	(2.48–3.39)	(6.9–7.5)