

tion of the technical monoglyceride mixture has concentrated in the extract the mono- and diesters of stearic acid present in the reaction mixture. The spread in iodine number of the two fractions was 28. The spread in iodine number would have been greater at a smaller amount extracted, or if there had been fewer diglycerides of mixed acids in the mixture.

### Summary

The experimental work has shown how molecular configurations of fatty acid glycerides affect urea complex formation. Extraction data indicate that urea will form a complex with glycerol monopalmitate, monostearate, and mono-oleate but not under these conditions with glycerol monolinoleate or monolinolenate from mixtures containing these monoesters. Data also indicate that it is easier for urea to form a complex with monoglycerides than with diglycerides.

In a mixture containing mono- and diglycerides of saturated and unsaturated fatty acids, urea will separate first on the basis of saturation, then secondly on the basis of the degree of esterification of the glycerol.

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## Composition of Liver Fats of Mature and Embryo Sharks (*Galeocerdo tigrinus*)

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THE EXCEPTIONALLY high contents of saturated acids in the liver fats from some Asiatic species of Elasmobranch fish (including sharks) have been observed by Tsujimoto (11, 12, 13) and by Wang and Kan (14). More recent studies conducted in this laboratory (8, 9, 10) have shown that the high content of saturated acids (*ca.* 40%) is characteristic of a group of Elasmobranch fish.

The present communication reports the comparative composition of the liver fats of mature and embryo sharks (*Galeocerdo tigrinus*). Both contained about 40% of saturated acids.

### Experimental

Two specimens of livers were supplied by the Government Oil Factory, Kozhikode, Calicut, Madras, from sharks caught in the Bay of Bengal. The first, designated as No. 1, was from the liver of a mother shark (120 in. in length). It weighed 49 lbs. and yielded 30% fat having a vitamin A potency of 9,970 i.u./g. The second specimen (No. 2) consisted of the livers of 12 male and 6 female embryo sharks (approximately 21 in. in length). The total weight of the embryo shark livers was 11 lbs. and yielded 45.5% fat having a vitamin A potency of 410 i.u./g.

*Fat No. 1.* A yield of 167 g. of mixed fatty acids (I. V. 88.5) was obtained from 180 g. of the phosphate free oil on saponification and removal of the unsaponifiable matter. These mixed acids were fractionated into three groups of varying unsaturation by the lithium (13) and lead salt alcohol (1) methods: (A) 71.0 g. (I.V. 9.5); (B) 64.0 g. (I.V. 132.1); and (C) 32.0 g. (I.V. 146.3).

Each fraction of the acids was esterified with methyl alcohol, and the esters were fractionated by use of the E.H.P. column (Longenecker's) (2) in the usual manner (8).

The composition of the mixed fatty acids (Table I) was calculated from the saponification equivalents

and iodine values of the ester fractions by the method described by Hilditch (1). The mean equivalent of each homologous ester group was found by interpolation and extrapolation of the unsaturation of each fraction.

*Fat No. 2.* The lead salt ether method (1) was used to fractionate 186.0 g. of the mixed acids (I.V. 57.7), obtained as described for Fat No. 1, into two fractions: (A) 70.0 g. (I.V. 3.3) and (B) 116.0 g. The composition of the mixed fatty acids is given in Table II.

TABLE I

Fat No. 1: Component Acids in Groups A, B, and C and Total Fatty Acids (all values as percentages of total)

Acids	A (42.5%)	B (38.3%)	C (19.2%)	Total (100.0%)
	%	%	%	%
Myristic.....	2.43	0.54	.....	2.97
Palmitic.....	23.46	1.67	.....	25.13
Stearic.....	13.06	0.76	.....	13.82
Arachidic.....	1.32	.....	.....	1.32
Unsaturated				
C <sub>14</sub> .....	.....	0.43(-2.0)	.....	0.43(-2.0)
C <sub>16</sub> .....	0.36(-2.0)	6.35(-2.0)	1.10(-2.0)	7.81(-2.0)
C <sub>18</sub> .....	1.28(-2.0)	18.43(-4.0)	3.91(-4.0)	23.62(-2.6)
C <sub>20</sub> .....	0.54(-2.0)	10.07(-5.1)	4.91(-6.8)	15.52(-5.6)
C <sub>22</sub> .....	.....	.....	9.26(-10.5)	9.26(-10.5)
Unsaponifiables.....	0.05	0.05	0.02	0.12

TABLE II

Fat No. 2: Component Acids in Groups A and B and Total Fatty Acids (all values as percentages of total)

Acids	A (37.63%)	B (62.37%)	Total (100.0%)
	%	%	%
Myristic.....	8.51	0.06	8.57
Palmitic.....	23.20	1.93	25.13
Stearic.....	4.32	0.73	5.05
Unsaturated			
C <sub>14</sub> .....	0.16(-2.0)	0.07(-2.0)	0.23(-2.0)
C <sub>16</sub> .....	0.70(-2.0)	17.25(-2.0)	17.95(-2.0)
C <sub>18</sub> .....	0.53(-2.0)	37.71(-2.1)	38.24(-2.1)
C <sub>20</sub> .....	.....	3.94(-4.4)	3.94(-4.4)
Unsaponifiables.....	0.21	0.68	0.89

### Discussion

The iodine value of the mixed fatty acids from the liver lipides of the mature shark was 88.5 as compared to 57.7 for those from the liver lipides of the shark embryo. This difference in the unsaturation of the two fats is more clearly seen from inspection of the amounts of the unsaturated acids present in them.

The fatty acids are broadly similar in composition in respect to the saturated acids. They contained 43.3 and 38.8% total saturated acids, 25.1 and 25.1% palmitic, 3.0 and 8.6% myristic, and 13.8 and 5.0% stearic acids, respectively. However there were differences in the actual contents of the several groups of unsaturated acids. Fat No. 1 contained the usual range of unsaturated acids including the C<sub>20</sub> acids (15.5%) and C<sub>22</sub> acids (9.3%). Fat No. 2 contained only 4% of C<sub>20</sub> and did not contain C<sub>22</sub> poly- or monoethylenic acids. The difference seems to have been made up by the higher content of C<sub>18</sub> (38.2%) and C<sub>16</sub> monoethenoid acids (18.0%).

The liver fats of Asiatic varieties of sharks that have so far been studied in this laboratory were found to contain large amounts of saturated acids, namely, those of *Galeocerdo rayneri* (8), *Carcharius melanopterus* (9, 10), and *Pristis cuspidatus* (9), which were found to contain about 40%, 32%, and 36% of saturated acids, respectively. This is in contrast to the normal "marine" animal type of fats, which contain about 20% of saturated acids. The liver fats of Elasmobranch fish were placed in three groups on the basis of the content of saturated acids by Tsujimoto (11). However, on the basis of his study of the *Carcharius gangeticus* liver oil (11), he envisaged a fourth group of liver fats containing about 50% of saturated acids. The liver fat of *Dasyatis akeji* studied by Wang and Kan (14) has been reported to contain about 64% of saturated acids. Lovern, from the study of the eels (3) and tunny fats (4), has observed that there is a definite tendency toward the deposition of a less unsaturated fat at higher temperatures. Although the findings of Lovern are not conclusive, it may be presumed safely that the Elasmobranch fishes of Asian waters deposit higher percentages of saturated acids in their depot fats.

The composition of the depot fat of an animal is influenced by its diet and also by the specific requirements of the animal in question. In the case of aquatic animals the ingested fat is the only source of depot fat. The difference in the depot fat composition from that of the ingested fat has been brought about by a process of metabolism which may be specific for a group of animals. This process may take the form of hydrogenation or dehydrogenation of the ingested fat. In the case of the fourth group of Elasmobranch fish liver fats Lovern (5) has visualized a process of simultaneous hydrogenation of the polyethylenic and monoethylenic derivatives of the preformed normal "marine" fat resulting in higher proportions of saturated acids.

Apart from a slightly higher content of myristic acid in the "embryo" fat, the contents of the saturated acids are almost the same in the two fats. However, as mentioned above, there are differences in the proportions of the various unsaturated acids as well as in the degree of unsaturation. The higher polyethenoids, a regular feature of the adult marine liver

fats, are absent in embryo liver fat. It seems that these acids, being more reactive, are utilized by the growing embryo. Such developments of specific fat compositions concurrently with the early developments of the species are indicated by the analysis of the body (blubber) fat of the foetus of a porpoise (6) and that of the developing eggs of salmon (7). In these cases a relatively high degree of unsaturation seems to be typical of the immature forms. This is in contrast to the findings of the present study. However it is not a safe procedure to apply results obtained on one species to other widely differing species, especially in connection with depot fat metabolism, which is often specialized. The fourth group of Elasmobranch fish shows evidence of fat hydrogenation. This process may be operative in a more pronounced degree in immature members. In that case the more saturated nature of the embryo liver fat may be expected. However the limited data available permit only preliminary conclusions.

### Summary

1. Two liver fats, one from the "mother" and the other from the "embryo" shark (*Galeocerdo tigrinus*), have been studied. Their component fatty acids are reported.
2. The mixed fatty acids from each of the fats were first resolved into groups of acids of varying unsaturation by the lithium salt acetone and/or lead salt alcohol methods. The methyl esters of each of these groups of acids were fractionated through the Longenecker's E.H.P. column.
3. The two fats are found to belong to the fourth group of Tsujimoto's classification of the Elasmobranch fish liver fats. The "mother" shark liver fat contains 43.3% saturated and 56.7% unsaturated acids. The "embryo" shark liver fat contains 39.1% saturated and 60.9% unsaturated acids. Palmitic acid was about 25% in both of the fats.
4. A possible explanation for the less unsaturation and the absence of higher polyethenoids in the "embryo" liver fat is given.

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