- Best, C. H., Hartroft, W. S., Lucas, C. C. & Ridout, J. H. (1955). *Brit. med. J.* p. 1439.
- Best, C. H., Lucas, C. C., Ridout, J. H. & Patterson, J. M. (1950). *J. biol. Chem.* **186**, 317.
- Best, C. H., Ridout, J. H., Patterson, J. M. & Lucas, C. C. (1951). *Biochem. J.* **48**, 448.
- Beveridge, J. M. R., Lucas, C. C. & O'Grady, M. K. (1944). *J. biol. Chem.* **154**, 9.
- Beveridge, J. M. R., Lucas, C. C. & O'Grady, M. K. (1945). *J. biol. Chem.* **160**, 505.
- Block, R. J. & Bolling, D. (1951). *The Amino Acid Composition of Proteins and Foods*, 2nd ed. Springfield, Ill.: C. C. Thomas.
- Cohn, H. P. & Berg, C. P. (1951). *Fed. Proc.* **10**, 172.
- Cole, A. S. & Scott, P. P. (1954). *Brit. J. Nutr.* **8**, 125.
- DeBey, H. J., Snell, E. E. & Baumann, C. A. (1952). *J. Nutr.* **46**, 203.
- Dick, F., jun., Hall, W. K., Sydenstricker, V. P., McCollum, W. & Bowles, L. L. (1952). *Arch. Path. (Lab. Med.)* **53**, 154.
- Grau, C. R. & Kamei, M. (1950). *J. Nutr.* **41**, 89.
- Hardin, J. O. & Hove, E. L. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 728.
- Harper, A. E., Benton, D. A. & Elvehjem, C. A. (1955). *Arch. Biochem. Biophys.* **57**, 1.
- Harper, A. E., Benton, D. A., Winje, M. E. & Elvehjem, C. A. (1954a). *J. biol. Chem.* **209**, 159.
- Harper, A. E., Benton, D. A., Winje, M. E. & Elvehjem, C. A. (1954b). *J. biol. Chem.* **209**, 171.
- Harper, A. E., Monson, W. J., Benton, D. A. & Elvehjem, C. A. (1953). *J. Nutr.* **50**, 383.
- Harper, A. E., Monson, W. J., Benton, D. A., Winje, M. E. & Elvehjem, C. A. (1954c). *J. biol. Chem.* **206**, 151.
- Hegsted, D. M., Mills, R. C., Elvehjem, C. A. & Hart, E. B. (1941). *J. biol. Chem.* **138**, 459.
- Kandutsch, A. A. & Baumann, C. A. (1953). *J. Nutr.* **49**, 209.
- Lucas, C. C. & Ridout, J. H. (1955). *Canad. J. Biochem. Physiol.* **33**, 25.
- McKittrick, D. S. (1947). *Arch. Biochem.* **15**, 133.
- Nino-Herrera, H., Harper, A. E. & Elvehjem, C. A. (1954). *J. Nutr.* **53**, 469.
- Roth, J. S., Allison, J. B. & Milch, L. J. (1950). *J. biol. Chem.* **186**, 113.
- Salmon, W. D. (1950). *Fed. Proc.* **9**, 369.
- Shils, M. E. & Stewart, W. B. (1954). *Proc. Soc. exp. Biol., N.Y.*, **85**, 298.
- Singal, S. A., Hazan, S. J., Sydenstricker, V. P. & Littlejohn, J. M. (1953). *J. biol. Chem.* **200**, 867.
- Stetten, D., jun. & Grail, G. F. (1942). *J. biol. Chem.* **144**, 175.
- Treadwell, C. R. (1948). *J. biol. Chem.* **176**, 1141.
- Treadwell, C. R., Tidwell, H. C. & Gast, J. H. (1944). *J. biol. Chem.* **156**, 237.
- Wretling, K. A. J. & Rose, W. C. (1950). *J. biol. Chem.* **187**, 697.

The Fatty Acid Composition of Indian Turtle Fat

BY S. P. PATHAK AND L. M. DEY

Department of Industrial Chemistry, Banaras Hindu University, India

(Received 20 September 1955)

As very little is known about the composition of the fats of amphibians, particularly of tropical waters, the present study on the fatty acid composition of the body fat of an Indian turtle was undertaken.

EXPERIMENTAL

The sample of body fat was from a turtle (*Erismochelonia imbricata*) of marine origin, caught in the Bay of Bengal (east coast of Madras State). It was kindly supplied by the Superintendent, Fisheries Technological Station, Kozhikode. The fat was refined and freed of phosphatides by the usual methods. It was then hydrolysed, and the mixed fatty acids thus obtained were resolved into three groups of

simpler mixtures of fatty acids by the lead salt-ethanol method (Hilditch, 1947) followed by further resolution of the soluble fraction by the lithium salt-acetone method (Tsujimoto, 1920; Tsujimoto & Kimura, 1923). Each group of the fatty acids was separately converted into the methyl esters, taking the usual precautions as recommended by Bjarnason & Meara (1944). The esters were then subjected to fractional distillation under high vacuum (0.1 mm. Hg) through an efficient electrically heated and packed column (Longenecker, 1937). The compositions of the subfractions were calculated from their respective iodine values and saponification equivalents according to the method recommended by Hilditch (1947). The mean unsaturations, as given in parentheses in Table 3, were determined as usual by interpolation and extrapolation

Table 1. Separation of the acids of turtle fat by the lead salt-ethanol and lithium salt-acetone methods

Group	Description	Wt.		Iodine value
		(g.)	(%)	
A	Lead salt-ethanol-insoluble	51.2	31.8	12.5
B	Lead salt-ethanol-soluble but lithium salt-acetone-insoluble	73.7	45.8	104.4
C	Lithium salt-acetone-soluble	36.0	22.4	166.8

from the respective ester fractions in each group. The final composition of the whole fat was then computed from these results and is given in Table 3.

RESULTS

Table 1 gives the fractional-crystallization data of the mixed fatty acids by the lead salt-ethanol and lithium salt-acetone methods; and Table 2 records the ester-fractionation results with saponification equivalents and iodine values.

The component acids of each group and of the whole fat are recorded in Table 3.

DISCUSSION

The present fat shows similarities to typical amphibian animal fats in its lower content of C_{16} unsaturated acids when compared with that of fish depot fats, and unsaturated C_{18} acids, mainly oleic acid, become the prominent individual group. Unsaturated acids of the C_{20} and C_{22} series are present up to about 15% in other amphibian fats but in the present case these rise to about 25%, which seems to be peculiar to this fat. The present fat also contains about 3% of unsaturated C_{24} acids, which again is unique for this class of fat. Another remarkable feature of the present fat is its high content (10%) of myristic acid. The presence of unusually large amounts of myristic acid has also been reported in a green-turtle fat studied by Tsujimoto (1937), and in other cases it has been found to be present to the extent of about 11%. The presence of about 15% of palmitic acid

Table 2. *Fractionation of methyl esters of turtle fat acids A, B and C*

Fraction	Wt. (g.)	Saponification equiv.	Iodine value
Methyl esters of A acids			
A1	3.14	242.2	1.2
A2	3.02	251.2	2.0
A3	3.06	257.3	2.0
A4	2.85	263.0	3.5
A5	3.17	268.3	3.9
A6	3.09	273.8	5.5
A7	2.92	283.6	8.2
A8	3.06	290.7	11.8
A9	3.09	298.1	15.9
A10	3.54	329.0*	53.5
Methyl esters of B acids			
B1	2.75	241.1	29.6
B2	2.95	266.3	65.6
B3	3.04	274.9	78.0
B4	3.16	280.4	82.8
B5	3.02	285.0	85.7
B6	3.31	294.0	91.6
B7	3.09	296.2	100.2
B8	3.21	298.6	105.2
B9	3.37	319.2	151.6
B10	3.19	340.1*	120.3
Methyl esters of C acids			
C1	2.85	269.1	95.9
C2	3.09	289.1	127.4
C3	3.34	310.2	170.8
C4	3.09	318.9	177.0
C5	2.92	325.4	177.9
C6	3.44	325.4	201.8
C7	2.24	332.2	188.9
C8	4.59	373.9*	107.8

* Equivalents of esters, freed from unsaponifiable matter: A10, 327.2; B10, 329.6; C8, 365.0.

Table 3. *Component acids in groups A, B, C and in the whole fat of turtle*

Acids	Component acids of groups (%)			Total (%)	Fatty acids in the whole fat, excluding non-saponifiables	
	A	B	C		% (w/w)	% (mol.)
Lauric	—	0.09	—	0.09	0.1	0.1
Myristic	7.22	2.95	—	10.17	10.2	12.3
Palmitic	12.52	2.50	—	15.02	15.1	16.2
Stearic	6.50	0.72	—	7.22	7.2	7.0
Arachidic	1.09	—	—	1.09	1.1	1.0
Behenic	0.05	—	—	0.05	Trace	Trace
Unsaturated						
C_{14}	0.10 (-2.0 H)	1.24 (-2.0 H)	—	1.34 (-2.0 H)	1.3	1.6
C_{16}	0.63 (-2.0 H)	9.32 (-2.0 H)	2.88 (-2.0 H)	12.83 (-2.0 H)	12.9	13.9
C_{18}	1.10 (-2.0 H)	19.07 (-2.3 H)	3.66 (-3.3 H)	23.83 (-2.4 H)	23.9	23.4
C_{20}	2.26 (-2.0 H)	8.36 (-4.2 H)	10.02 (-4.8 H)	20.64 (-4.3 H)	20.7	18.5
C_{22}	0.31 (-2.0 H)	1.40 (-5.0 H)	2.89 (-6.0 H?)	4.60 (-5.4 H?)	4.6	3.8
C_{24}	—	—	2.85 (-10.0 H?)	2.85 (-10.0 H?)	2.9	2.2
Unsaponifiable	0.02	0.15	0.10	0.27	—	—

is quite normal, since other varieties of turtle fats contain about 15–22% of this acid (Table 4). The stearic acid content (7.2%) in the present fat is also not abnormal.

Among the unsaturated acids, the turtle fat now described resembles those studied by others in its content of 1.3 and 12.9% of monoethylenic C_{14} and C_{16} acids respectively. Decrease in the unsaturated

The deviation therefore, in the fatty acid composition of the present sample of turtle fat from those studied by others may be due to its being from an animal belonging to a different species, or to the fact that the present fat was obtained from an animal living in its natural surroundings (the sea), whereas others might have been in captivity for a long time.

Table 4. *Constituent fatty acids (% w/w) of the fats of a few species of turtle*

Acids	Turtle*	Turtle†	Turtle‡	Turtle§
Decanoic	0.2	—	—	—
Lauric	13.3	14.2	—	0.1
Myristic	10.6	7.2	6.6	10.2
Palmitic	17.0	15.2	21.8	15.0
Stearic	4.1	6.8	15.5	7.2
Arachidic	—	—	1.9	1.1
Behenic	—	—	—	0.1
Unsaturated				
C_{14}	1.3 (-2.0 H)	2.6 (-2.0 H)	3.5 (-2.0 H)	1.3 (-2.0 H)
C_{16}	7.8 (-2.0 H)	10.9 (-2.0 H)	18.0 (-2.0 H)	12.9 (-2.0 H)
C_{18}	39.6 (-2.2 H)	39.4 (-2.0 H)	31.4 (-3.7 H)	23.9 (-2.4 H)
C_{20}	6.1 (-6.3 H)	0.2 (-? H)	1.3 (-8.6 H)	20.7 (-4.3 H)
C_{22}	—	—	—	4.6 (-5.4 H)
C_{24}	—	—	—	2.9 (-10.0 H?)

* Green & Hilditch (1938).

§ Present study.

† Ogata & Minato (1940).

|| Also contains 3.5% of hexanoic acid.

‡ Giral & Marquez (1948).

C_{18} acid content with the increase in the amounts of higher unsaturated acids of the C_{20} , C_{22} and C_{24} series is striking and makes the present fat stand apart from the other turtle fats. Such a deviation in fatty acid composition may be due to various factors that govern the fatty acid compositions of fats in animals. The presence of a high proportion (66.3%) of unsaturated acids, which is typical of marine animal fats, may be due to the marine origin of the present animal. Another factor which governs fatty acid composition is the life habits of animals. A marked difference in unsaturation was observed in fats from wild and tame rabbits (Lewkowitsch, 1922). By comparing the compositions of two crocodile fats, given by Gunstone & Russell (1954), in which *Crocodylus niloticus* fat was obtained from an animal in its natural state and *Crocodylus porosus* fat was obtained from an animal kept in captivity for several years, we find that the animal living in its natural state contained larger amounts of unsaturated C_{16} and C_{20-22} acids and lower amounts of C_{18} acids with a higher degree of unsaturation than those from an animal kept in captivity. The species difference may also be a factor.

SUMMARY

1. The composition of the body fat from a turtle (*Erismochelies imbricata*) has been studied. Lead salt-ethanol and lithium salt-acetone methods were adopted for preliminary separation of the mixed fatty acids into groups differing in unsaturation. The compositions of the resulting fractions were studied by the ester-fractionation procedure.

2. Significant differences have been found in the proportions of saturated and unsaturated acids, when compared with those of other amphibian animal fats.

3. The high content of unsaturated acids, which is typical of marine animal fats, is remarkable in the fat described. Decrease in the unsaturated C_{18} acid content with the increase in the amounts of higher unsaturated acids of C_{20} , C_{22} and C_{24} series makes the fat stand apart from others in this category.

4. Possible reasons for this deviation in fatty acid composition are discussed.

The authors wish to express their cordial thanks to Mr R. Venkatraman, Superintendent, Fisheries Technological Station, Kozhikode, Madras, for supplying the sample of oil which made the present work possible.

REFERENCES

- Bjarnason, O. B. & Meara, M. L. (1944). *J. Soc. chem. Ind., Lond.*, **63**, 61.
- Giral, F. & Marquez, A. (1948). *Arch. Biochem.* **16**, 187.
- Green, T. G. & Hilditch, T. P. (1938). *Biochem. J.* **32**, 681.
- Gunstone, F. D. & Russell, W. C. (1954). *Biochem. J.* **57**, 462.
- Hilditch, T. P. (1947). *The Chemical Constitution of Natural Fats*, 2nd ed. London: Chapman and Hall.
- Lewkowitsch, J. (1922). *Technology of Oils, Fats and Waxes*, 2nd ed., vol. 2. London: Macmillan and Co. Ltd.
- Longenecker, H. E. (1937). *J. Soc. chem. Ind., Lond.*, **56**, 199T.
- Ogata, A. & Minato, A. (1940). *J. pharm. Soc., Japan*, **60**, 76.
- Tsujimoto, M. (1920). *J. Soc. chem. Ind., Japan*, **23**, 1007.
- Tsujimoto, M. (1937). *J. Soc. chem. Ind., Japan*, **40**, 185B.
- Tsujimoto, M. & Kimura, K. (1923). *J. Soc. chem. Ind., Japan*, **26**, 891.

The Nature of the Effect of Ammonium Sulphate on the Biosynthesis of Ascorbic Acid in Plants

BY K. SIVARAMA SASTRY AND P. S. SARMA

University Biochemical Laboratory, University of Madras, Madras 25, India

(Received 9 August 1955)

Ever since Ray (1934) showed that excised pea embryos could synthesize ascorbic acid on a semi-solid medium containing hexoses, it has been known that ascorbic acid may be formed from carbohydrate sources. However, the first direct evidence for the hypothesis was provided by Jackel, Mosbach, Burns & King (1950), who demonstrated a conversion of uniformly labelled [^{14}C]glucose into uniformly labelled ascorbic acid in the rat. Previous work based essentially on the increased excretion of ascorbic acid when various precursors were administered had not been conclusive, owing to the well-known stimulatory effect of various substances, some of which could not, by their very nature, be precursors (Longenecker, Musulin, Tully & King, 1939). The work of Jackel

et al. (1950) provided convincing evidence that glucose served as a precursor for this vitamin.

An analogous position is found when plants are considered. Vitamins of the B group and a number of amino acids, as well as intermediates of the citric acid cycle, have all been found to stimulate ascorbic acid synthesis (Bharani, Shah & Sreenivasan, 1953). Hence it was desirable to obtain direct evidence for the conversion of glucose into ascorbic acid in plants as well, by means of an isotopic technique.

That glucose is the most likely precursor has been shown by the work of Isherwood, Chen & Mapson (1954). Their work has indicated that glucose may be converted into ascorbic acid according to the sequence shown in Fig. 1. The

Fig. 1. Conversion of glucose into ascorbic acid.