

EFFECT OF DIET ON DEPOT FATTY ACID COMPOSITION IN THE GREEN TURTLE *CHELONIA MYDAS*

JEANNE D. JOSEPH, ROBERT G. ACKMAN* and GLORIA T. SEABORN

Charleston Laboratory, National Marine Fisheries Service, NOAA, Charleston, SC 29412, USA and

*Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, Halifax, NS B3J 2X4,
Canada

(Received 18 April 1984)

Abstract—1. High-resolution GLC was used to compare fatty acid compositions of fatty tissues and rendered oils from wild and cultured green turtles with that of their dietary lipids.

2. While there was great similarity in composition of fats from cultured turtles and their feeds, there was no such relationship between the composition of wild turtle fats or oils and two seagrasses analyzed.

3. Regardless of dietary regime, 12:0 and 14:0 comprised two of the principal fatty acids in all green turtle fats and oils examined.

INTRODUCTION

Due to long-term over-exploitation, animal predation, and more recently, to coastal property development and incidental capture in commercial fishing operations, many species of marine turtles inhabiting coastal waters of the USA are in danger of extinction. In recognition of this fact, the Congress of the United States passed the Endangered Species Act in 1973 to reduce exploitation and promote conservation of sea turtles as well as other endangered plant and animal species. Under the provisions of the Act, it is unlawful to import these species, their parts, or products without proper scientific permits. While cosmetic products purported to contain turtle oils are commercially available, the constituent turtle oils could be of terrestrial or freshwater turtles not protected by the Act. Therefore, law enforcement personnel of the National Marine Fisheries Service, US Customs Service and US Fish and Wildlife Service have need of forensic methods for positive identification of marine turtle oils in cosmetics.

Our strategy for development of the required methodology was based upon the report of significant differences in fatty acid composition of depot fats from marine and freshwater turtles (Ackman *et al.*, 1971a). However, in order to use fatty acid composition as the distinguishing characteristic, it was first necessary to determine the extent of individual variation in composition which could be expected by analyzing multiple samples of authenticated turtle oils. During this phase of our work, it became obvious that the fatty acid composition of green turtle oils was variable and depended upon the dietary history of the turtles from which the oils were derived. In this report we describe the fatty acid composition of: (1) depot fats of three adult green turtles, two wild and one aquarium-reared; (2) a number of rendered oils of both wild and pen-reared adult green turtles; and (3) dietary components known or believed to be important in influencing the fatty acid composition of the turtle depot fats.

MATERIALS AND METHODS

Samples analyzed in this study were obtained from a variety of sources. Depot fat tissues were obtained from a wild female turtle captured at sea between the Yucatan Peninsula and Key West, FL in 1973, and from an adult albino green turtle (sex unknown) held in captivity until its death in 1980 at the Miami (FL) Sea Aquarium. This turtle was fed an unknown species of herring as a significant component of its diet (F. Berry, Southeast Fisheries Center, Miami, FL, pers. commun.). A depot fat sample from an adult turtle taken in Hawaiian coastal waters was supplied by G. H. Balazs (Hawaii Institute of Marine Biology, Kanohe, HI). Rendered oils from pen-reared turtles and a sample of their commercial extruded ration were the gift of Grand Cayman Farm, Ltd. Rendered oils were also obtained from a native market in Panama and from commercial sources in Italy and the United Kingdom. The river herring, *Alosa aestivalis*, was obtained during its annual spring migration up the Cooper River, near Charleston, SC. New growth of the seagrasses, *Thalassia testudinum* and *Syringodium filiforme*, were collected near Panama City, FL, frozen and shipped with dry ice to the Charleston Laboratory for analysis.

As the data reported in this paper have been acquired in two different laboratories over a period of several years, methods of sample preparation and analysis have differed somewhat, but have been based upon established methodology. Depot fats of the Caribbean wild female and the Hawaiian specimen were extracted in Halifax by the Bligh and Dyer (1959) method. Those of the aquarium-reared adult were extracted at the Charleston Laboratory with acetone and the extracted fat transferred to hexane after addition of water. Fatty acid methyl esters of all turtle fat extracts and rendered oils were prepared by transmethylation (Christopherson and Glass, 1969) for analysis by gas-liquid chromatography (GLC). The river herring, seagrasses and commercial feed of the pen-reared turtles were extracted by the Bligh and Dyer (1959) method and transesterified by the method of Metcalfe *et al.*, (1966). Esters of the seagrasses were purified by thin-layer chromatography (TLC) prior to GLC.

Fatty acid methyl esters were analyzed on stainless-steel 50 m columns coated with butanediol succinate (BDS) or Apiezon-L (Ap-L) or on a flexible fused silica 30 m column coated with Carbowax 20 M, installed in Perkin-Elmer* instruments in the Halifax Laboratory, as described previously (Ackman *et al.*, 1971a; Hooper and Ackman, 1970). In Charleston, flexible fused silica 50 m columns coated with Silar 5-CP and a 50 m stainless-steel column coated with Ap-L, installed in Hewlett-Packard equipment, were used.

*Mention of commercial products or companies does not constitute endorsement by the National Marine Fisheries Service, NOAA.

Table 1. Fatty acid composition, in weight percent, of crude and refined pen-reared green turtle oils and compounded turtle feed

Sample:	Crude oil	Refined oil	Feed
Fatty acids			
Saturates			
10:0	0.11	0.13	0.05
12:0	9.90	9.83	0.09
13:0	0.02	0.05	<0.01
14:0	6.94	7.00	1.36
15:0	0.14	0.14	0.16
16:0	4.31	14.94	20.23
17:0 ^a	0.16	0.16	0.49
18:0	4.58	4.69	10.87
20:0	0.06	0.05	0.24
Monoenes			
14:1 ω 5	0.43	0.45	0.18
16:1 ω 9	0.50	0.53	0.19
16:1 ω 7	6.25	6.19	1.80
16:1 ω 5	0.09	0.04	0.09
18:1 ω 9	32.33	31.78	34.54
18:1 ω 7	3.58	3.82	2.08
18:1 ω 5	0.17	0.16	0.19
20:1 ω 11	0.05	0.05	<0.01
20:1 ω 9	0.59	0.49	0.54
20:1 ω 7	0.07	0.05	<0.01
Polyenes			
18:2 ω 6	14.14	12.67	24.20
20:2 ω 6	0.27	0.27	0.16
18:3 ω 6	0.17	0.14	<0.01
18:3 ω 3	1.13	0.92	1.58
20:3 ω 6	0.36	0.31	<0.01
20:3 ω 3	0.03	0.05	<0.01
18:4 ω 3	0.18	0.15	<0.01
20:4 ω 6	0.61	0.49	0.11
20:4 ω 3	0.06	0.03	<0.01
22:4 ω 6	<0.01	<0.01	<0.01
20:5 ω 3	0.17	0.06	0.07
22:5 ω 6	<0.01	<0.01	<0.01
22:5 ω 3	0.35	<0.01	<0.01
22:6 ω 3	0.24	<0.01	<0.01

^aIncludes 7-methyl-7-hexadecenoate and phytanate.

Identification of components was based upon comparisons of retention times of the esters with those of authentic standards or secondary standards, analyses of esters separated into fractions according to degree of unsaturation by AgNO₃/TLC, and upon analysis of hydrogenated samples. Chromatograms were quantified by electronic integration. Listing of weight percent composition to two decimal places permits comparison of minor components but does not imply an accuracy greater than $\pm 5\%$ for major components ($> 5\%$), $\pm 10\%$ for components in the 1–5% range, and $\pm 50\%$ for minor components ($< 1\%$).

RESULTS

Cultured turtle oils and feed lipids

The fatty acid composition of depot fats from cultured turtles clearly showed the influence of their dietary fatty acids. The fatty acid arrays* of both crude and refined oils from pen-reared turtles as well as those of their feed (Table 1) were dominated by 16:0, 18:1 ω 9 and 18:2 ω 6, the latter a fatty acid not biosynthesized by higher animals (Fulco, 1977). However, the turtle oils also contained substantial

*Shorthand notation specifies the number of carbon atoms and double bonds in the molecule. The number following the Greek letter " ω " indicates the position of the ultimate double bond relative to the methyl group of the molecule. A double bond position preceded by the Greek letter " Δ " is determined from the polar (carboxyl) group of the molecule. All double bonds are *cis* in geometry except those designated *trans*.

percentages of 12:0 and 14:0 which were present only in small amounts in the feed. These latter two fatty acids were also prominent in fats of the aquarium-reared turtle (Table 2). Unlike the pen-reared turtle oils, the fat of the aquarium-reared turtle contained little 18:2 ω 6, approximately half as much 18:1 ω 9 and substantial amounts of 20 and 22 carbon monoenes, particularly 20:1 ω 9 and 22:1 ω 11 + 13, and the characteristic marine polyunsaturates, 20:5 ω 3 and 22:6 ω 3. These are features shared with the depot fats of clupeids and a number of other carnivorous fishes of the northeast Atlantic coast (Ackman *et al.*, 1963; Ackman and Burgher, 1964; Ackman and Eaton, 1966, 1971; Ackman and Ke, 1968). The fatty acid composition of lipids of the river herring, *A. aestivalis*, determined in the Charleston Laboratory, was selected for comparison with that of the aquarium-reared turtle depot fat, although any of several published analyses of clupeid fatty acids (Ackman and Eaton, 1966; Ackman *et al.*, 1967, 1975a) would be equally suitable. Although 14:0 was present in modest proportion in the herring lipids, the percentage of 12:0 was inconsequential.

Wild turtle oils and seagrass lipids

The fatty acid composition of the Caribbean wild turtle depot fat sample was very similar, qualitatively

Table 2. Fatty acid composition, in weight percent, of aquarium-reared albino green turtle depot fat and lipids of the river herring, *Alosa aestivalis*

Sample:	Turtle depot fat	Herring lipids
Fatty acids		
Saturates		
10:0	0.14	0.11
12:0	5.39	0.04
13:0	0.04	0.02
14:0	7.25	5.06
TM Δ 9	0.11	0.28
15:0	0.29	0.42
16:0	13.33	15.45
17:0 ^b	0.47	0.80
18:0	3.23	2.98
20:0	0.21	0.07
Monoenes		
14:1 ω 5	0.49	0.21
16:1 ω 9	0.26	0.20
16:1 ω 7	9.09	2.88
16:1 ω 5	0.21	0.21
18:1 ω 11	0.71	0.97
18:1 ω 9	18.77	10.47
18:1 ω 7	3.26	2.19
18:1 ω 5	0.36	0.44
20:1 ω 11	0.62	1.65
20:1 ω 9	5.83	12.36
20:1 ω 7	0.36	0.38
20:1 ω 5	0.04	0.11
22:1 ω 11+13	5.18	13.46
22:1 ω 9	0.61	1.06
22:1 ω 7	0.08	0.12
Polyenes		
18:2 ω 6	0.89	1.35
20:2 ω 6	0.10	0.34
18:3 ω 6	0.04	0.06
18:3 ω 3	0.47	1.03
20:3 ω 6	0.06	0.06
20:3 ω 3	0.05	0.18
18:4 ω 3	0.53	1.56
20:4 ω 6	0.50	0.51
20:4 ω 3	0.59	1.07
22:4 ω 6	0.19	0.24
20:5 ω 3	5.31	5.26
22:5 ω 6	<0.01	0.09
22:5 ω 3	1.32	1.39
22:6 ω 3	11.10	10.84

^a4,8,12-Trimethyltridecanoate.

^bIncludes 7-methyl-7-hexadecenoate and phytanate.

Table 3. Fatty acid composition, in weight percent, of rendered wild green turtle oils from Panama and Italy (Somalia), two wild adult depot fats, and lipids of two seagrasses, *Thalassia testudinum* and *Syringodium filiforme*

Origin:	Panama	Somalia	Hawaii	Caribbean Sea		
Sample:	Oil	Oil	Depot Fat	Depot Fat	Sea Grasses	
					<i>T. testudinum</i>	<i>S. filiforme</i>
Fatty Acids						
Saturates						
10:0	0.15	0.18	0.17	0.63	<0.01	0.43
12:0	14.00	13.13	12.11	7.26	0.05	0.12
13:0	0.05	0.03	0.18	0.04	0.15	<0.01
14:0	9.99	9.79	8.15	17.08	0.80	1.36
15:0	0.09	0.13	0.47	0.18	0.26	0.21
16:0	17.53	16.82	13.82	22.17	28.88	20.23
17:0 ^a	0.20	0.13	0.46	0.55	0.28	0.43
18:0	4.24	5.07	4.61	4.74	3.63	1.83
20:0	0.06	0.07	0.47	0.11	0.03	0.50
Monoenes						
14:1 ω 5	0.64	0.68	0.62	0.92	0.09	0.07
t 16:1 ω 10	0.03	0.04	0.47	<0.01	<0.01	<0.01
16:1 ω 9	0.85	0.83	0.25	0.50	0.23	0.18
16:1 ω 7	8.02	7.96	5.55	10.58	4.07	5.27
16:1 ω 5	0.06	0.02	0.23	0.22	0.13	0.10
18:1 ω 11	0.15	0.12	0.30	<0.01	<0.01	<0.01
18:1 ω 9	31.18	30.92	25.22	26.22	3.47	4.46
18:1 ω 7	3.80	4.04	4.00	4.70	2.79	0.96
18:1 ω 5	0.15	0.16	0.25	0.05	0.03	0.02
20:1 ω 11	0.08	0.06	0.20	0.05	0.02	0.04
20:1 ω 9	0.45	0.41	0.88	0.39	0.27	0.14
20:1 ω 7	0.05	0.12	0.14	0.18	0.04	0.02
20:1 ω 5	<0.01	<0.01	0.02	0.01	<0.01	<0.01
22:1 ω 11+13	<0.01	<0.01	0.08	0.01	0.01	<0.01
22:1 ω 9	0.04	0.04	0.44	0.05	0.05	<0.01
22:1 ω 7	<0.01	<0.01	0.07	0.01	0.01	<0.01
Polyenes						
C 16	0.02	0.03	0.59	<0.01	0.97	0.57
18:2 ω 9?	0.07	0.05	0.02	0.14	<0.01	<0.01
18:2 ω 6	1.67	1.16	0.58	0.07	19.89	16.73
20:2 ω 9?	0.28	0.23	0.02	0.14	<0.01	<0.01
20:2 ω 6	0.06	0.04	0.22	0.14	0.18	0.21
18:3 ω 6	0.09	0.08	0.09	<0.01	0.03	0.27
18:3 ω 3	1.05	1.15	0.24	0.13	27.92	31.31
20:3 ω 9?	0.28	0.16	0.05	0.10	<0.01	<0.01
20:3 ω 6	0.15	0.22	0.13	0.02	0.05	0.10
20:3 ω 3	0.07	0.05	0.07	<0.01	0.17	0.12
18:4 ω 3	0.01	0.10	0.12	0.04	1.12	0.50
20:4 ω 6	0.42	0.79	1.30	0.33	0.66	1.18
20:4 ω 3	0.04	0.11	0.25	0.02	<0.01	0.09
22:4 ω 6	0.32	0.37	1.28	0.04	<0.01	<0.01
20:5 ω 3	0.16	0.12	0.89	0.09	0.31	3.40
22:5 ω 6	0.05	0.09	1.48	0.01	<0.01	<0.01
22:5 ω 3	0.80	1.11	2.42	0.05	<0.01	0.05
22:6 ω 3	0.20	0.31	7.26	0.01	<0.01	0.47

^aIncludes 7-methyl-7-hexadecenoate and phytanate.

and quantitatively, to that of the rendered oils from Panama and Italy, the latter of Somalian origin (Table 3). A cover letter received with this oil provided by Esperis S.P.A. (Milan, Italy) stated that the oil was from *Chelonia imbricata*, the "common big turtle" of Somalia (P. Rossi, pers. commun.). The fatty acid composition of this oil was identical to that of the composite green turtle oil from the Caribbean. The composition of these three samples was quite different from that of the cultured turtles and none bore any resemblance to that of the seagrasses, *T. testudinum* and *S. filiforme*. The most important fatty acids, quantitatively, in the wild turtle oils were 12:0, 14:0, 16:0 and 18:1 ω 9. Neither 18:2 ω 6 nor 18:3 ω 3, totaling 47.81% and 48.34% in *T. testudinum* and *S. filiforme*, respectively, were present in appreciable amounts (< 2%) in the wild turtle oils. Our analysis of *T. testudinum* agreed well with respect to the major components with that reported previously by Maurer and Parker (1967). The fatty acid composition of the seagrasses was similar, in most respects, to that of the

Australian seagrasses, *Posidonia australis* and *Heterozostera tasmanica* (Nichols *et al.*, 1982). However, we did not observe *trans* 16:1 ω 13, common in photosynthetic tissues, in the lipids of *T. testudinum* or *S. filiforme*.

As in the Caribbean fat sample and rendered oils, 12:0, 14:0, 16:0, and 18:1 ω 9 were prominent in the Hawaiian fat sample (Table 3). In addition, however, this sample contained significantly more 20 and 22 carbon polyenes and even less 18:2 ω 6 and 18:3 ω 3 than the small percentages found in the Caribbean fat and rendered oils.

Other rendered turtle oils

The geographic origin of the rendered green turtle oil from the United Kingdom (Table 4) is not known but the high percentage of 18:2 ω 6 and the general similarity of its composition to that of the pen-reared turtle oils (Table 1) leads us to believe that this oil originated from cultured turtles fed a diet similar to that used at the Grand Cayman Farm, Ltd.

Table 4. Fatty acid composition, in weight percent, of a commercial rendered green turtle oil from the United Kingdom

Fatty acid	Percent	Fatty acid	Percent
Saturates		Monoenes	
10:0	0.40	20:1 ω 7	0.14
12:0	10.93	22:1 ω 11+13	0.07
13:0	0.02	22:1 ω 9	0.05
14:0	6.82	Polyenes	
15:0	0.18	14:3 ω 3	14.39
16:0	13.51	18:2 ω 6	0.16
17:0 ^a	0.37	18:3 ω 6	0.05
18:0	5.70	18:3 ω 3	1.20
20:0	0.03	20:3 ω 6	0.17
Monoenes		20:3 ω 3	0.04
14:1 ω 5	0.42	18:4 ω 3	0.11
16:1 ω 9	0.29	20:4 ω 6	0.41
16:1 ω 7	5.76	20:4 ω 3	0.10
16:1 ω 5	0.22	22:4 ω 6	0.25
18:1 ω 9	26.39	20:5 ω 3	1.31
18:1 ω 7	5.50	22:5 ω 6	0.05
18:1 ω 5	0.71	22:5 ω 3	1.20
20:1 ω 11	0.02	22:6 ω 3	1.91
20:1 ω 9	0.42		

^aIncludes 7-methyl-7-hexadecenoate and phytanate.

DISCUSSION

Transfer of dietary fatty acids through the marine food web has been suggested in recent years by the results of a number of laboratory and field studies of phyto- and zooplankton, invertebrates and teleost fishes (Ackman *et al.*, 1970, 1975b; Lee *et al.*, 1971; Mayzaud *et al.*, 1976; Bottino, 1974; Paradis and Ackman, 1976a,b, 1977; Pascal and Ackman, 1976; Bishop *et al.*, 1976). Although published data on lipids and fatty acids of marine reptiles are limited, two fatty acids believed to be of invertebrate origin, *trans* 16:1 ω 10 and 20:4 ω 6, have been reported in fats of three species of carnivorous turtles, the leatherback, *Dermochelys coriacea*, the Kemp's ridley, *Lepidochelys kempii*, and the loggerhead, *Caretta caretta* (Hooper and Ackman, 1970; Ackman *et al.*, 1971a, 1972). However, there are no published records, based upon modern technology, of the fatty acids of the green turtle, *Chelonia mydas*. Although this species readily consumes animal matter in captivity, as an adult in the wild it is reported to be, preferentially, a herbivore (Mortimer, 1982). Throughout most of its world-wide range, the green turtle forages on seagrasses or, in their absence, on algae (Mortimer, 1982; Bjorndal, 1982; Balazs, 1982). In the Caribbean Sea, 80–90% of the green turtle's diet consists of new growth of the seagrass, *T. testudinum* but *S. filiforme* is also considered important in the diet of this species in some regions (Mortimer, 1982). However, green turtles of the Hawaiian Archipelago are reported to feed primarily on algae (Mortimer, 1982).

Mortimer (1982) investigated the possibility that commensal plant or invertebrate growth on the seagrass blades might contribute significantly to the nutrition of the Caribbean green turtle. She found that only about 5% of the *Thalassia* present in the stomachs of green turtles consisted of older blades. This observation agreed with that of Bjorndal (1980) that the green turtle forages on new growth. Thus, epiphytic organisms, largely restricted to older blades, contribute little to the Caribbean green turtle's nutrition. We found it relatively simple to

select new blades of *T. testudinum* for analysis but much more difficult to sample new growth of *S. filiforme* because of its narrow blade width. Consequently, a greater proportion of this sample consisted of older growth. The presence of commensal organisms may explain the higher levels of 20:5 ω 3 in *S. filiforme* lipids (Table 3), as this fatty acid is prominent in marine phytoplankton (Ackman *et al.*, 1968; Joseph, 1975) and filter feeders (Ackman *et al.*, 1970, 1974a,b). Thus, it might also be expected to be an important fatty acid of seagrass epifauna (Lewis and Hollingsworth, 1982).

Because the marine seed plants on which the green turtle grazes form dense submarine stands only in relatively calm waters, but nesting occurs on exposed shores where wave action has created dunes, feeding grounds and nesting beaches of this species are often widely separated geographically (Carr, 1982). The results of world-wide tagging studies indicate that some green turtle populations migrate as far as 3000 km from feeding grounds to nesting beaches (Meylan, 1982). Consequently, the migratory green turtle may be subjected to periodic food deprivation in the wild. In both laboratories, small percentages of tentatively identified ω 9 polyunsaturated fatty acids were detected in wild turtle fats and oils (Table 3). These compounds are biosynthesized by some vertebrates from 18:1 ω 9 when deprived of the essential fatty acid, 18:2 ω 6 (Holman, 1977). This process occurs in a biochemical attempt by the animal to satisfy the requirement for polyunsaturates, particularly 20:4 ω 6, in cell membranes. If identified correctly, the presence of ω 9 polyunsaturates in the wild green turtle oils might indicate a partial essential fatty acid deficiency.

One of the rendered oils included in this study, although obtained from a company in Italy, was imported by that firm from Somalia. The specific name, *Chelonia imbricata*, supplied with this oil, is not in use at present but is one of 12 archaic specific names of the hawksbill turtle, *Eretmochelys imbricata* (Witzell, 1983). Both green and hawksbill turtles nest on the Somalian coast. However, since the carapace length of the mature green turtle is some 20–30 cm greater than that of the hawksbill and its population density in this region is much greater (W. Witzell, Miami Laboratory, National Marine Fisheries Service, Southeast Fisheries Center, Miami, FL, pers. commun.), we have little doubt that the "common big turtle" of Somalia is probably *C. mydas*. There is no question that the fatty acid composition of this oil is indistinguishable from that of the composite Caribbean green turtle oil (Table 3). In contrast, a composite hawksbill turtle oil from the Caribbean contained only 0.43% 12:0 (Joseph and Seaborn, unpub. data). All of the biological and biochemical evidence that we have on this oil, therefore, indicates that it is largely, if not exclusively, green turtle oil.

The similarity in fatty acid composition of this oil from the Indian Ocean to that of an oil rendered by a native turtle fisherman in Panama (Table 3) suggests that certain fatty acids are characteristic of green turtle oils. The presence of substantial amounts of 12:0 and 14:0 were observed in all of the turtle fats and rendered oils, regardless of dietary regimes. Hilditch and Williams (1964) compiled published

data on fatty acids of the green turtle, commented on the high percentage of 12:0, and suggested that it was of dietary origin. However, the relative absence of this fatty acid in any of the dietary lipids analyzed in this study indicates its biogenic origin in the turtle. In a study of digestion in the green turtle, Bjorndal (1979, 1980, 1982) reported that this reptile digests cellulose as efficiently as ruminants. Volatile, short-chained fatty acids, acetic, butyric and some propionic, are produced as a result of microbial fermentation in the hindgut, and are absorbed, largely, in the caecum and large intestine. Acetic acid was reported to be by far the major fermentation product. Thus, this acid probably serves as the raw material for 12:0 and 14:0 biosynthesis by way of acetyl-CoA condensation. Microbial biohydrogenation of 18:2 ω 6 and 18:3 ω 3 in the gut, with subsequent β -oxidation of 18:0 to 12:0 and 14:0 offers an alternative possibility. Either process would provide energy and also satisfy a possible requirement for a depot fat of low melting point but without a high proportion of unsaturated fatty acids.

The fat of the leatherback turtle also has comparable proportions of 12:0 and 14:0 (Ackman *et al.*, 1971a). It is believed that this species feeds mostly on jellyfishes (see below) which are extremely low in lipid content (Sipos and Ackman, 1968). Since the extracellular mesogloea of jellyfishes consists of mucopolysaccharides in association with proteins (Chapman, 1966), dietary jellyfishes contribute energy largely in the form of carbohydrate which, by processes similar to those described above, can be converted to saturated fatty acids. Therefore it seems probable that the source of 12:0 and 14:0 in leatherback oils is jellyfish carbohydrate.

In view of the low fat content of the seagrasses, 0.2% of the wet weight in both species, and the low percentages of 18:2 ω 6 and 18:3 ω 3 in the wild turtle oils, it seems doubtful that the wild green turtle of the Caribbean deposits any appreciable amount of dietary fat. In particular, the data suggest that these turtles readily catabolize 18:2 ω 6 and 18:3 ω 3 in excess of their biochemical needs. Furthermore, it appears that in the wild, the green turtle biosynthesizes and deposits those fatty acids which most, if not all, animals are able to synthesize by acetyl-CoA condensation; 12:0, 14:0, 16:0, and 18:0. Oleic acid (18:1 ω 9) is readily synthesized by oxidative desaturation of the parent acid, 18:0 (Gurr and James, 1971). These metabolic transformations seem to offer the most feasible explanation for the observed dissimilarities in composition of Caribbean green turtle oils and lipids of their forage.

Mortimer (1982) has summarized numerous reports of algae in the diet of green turtles from the mid-Pacific Ocean and Balazs (1982) has also noted that they feed not only on algae but also "voraciously" upon jellyfishes (Phylum Cnidaria). Unlike the seagrasses, lipids of most of the Rhodophyceae that have been analyzed contain little 18:2 ω 6 and 18:3 ω 3, but, along with the Phaeophyceae, are good dietary sources of 20:4 ω 6 and 20:5 ω 3 for marine herbivores (Ackman and McLachlan, 1977; Ackman, 1982; Jamieson and Reid, 1972). Chlorophyceae, on the other hand, provide predators with little dietary 20:4 ω 6 or 20:5 ω 3 but significant amounts of 16 and

18 carbon ω 3 polyenes (Ackman and McLachlan, 1977; Jamieson and Reid, 1972). Both benthic and pelagic cnidarians contain substantial percentages of 20:4 ω 6 and its longer-chained successor acids, 22:4 ω 6 and 22:5 ω 6 (Sipos and Ackman, 1968; Hooper and Ackman, 1971; Joseph, 1979). Analyses of lipids of the jellyfishes, *Cyanea capillata* (Sipos and Ackman, 1968), *Stomolophus meleagris* and *Chrysaora quinquecirrha* (Joseph, 1979) have indicated that some pelagic Cnidaria are also rich in 20:5 ω 3, 22:5 ω 3, and 22:6 ω 3. In addition to these commonly-occurring polyenes, the more unusual monoene, *trans* 16:1 ω 10, has been reported in lipids of the scyphozoan jellyfish *Aurelia aurita* Lamarck (Hooper and Ackman, 1972) and the Portuguese-man-of-war, *Physalia physalis* (Stillway, 1976) as well as in a number of cnidarian predators. These predators include three species of carnivorous sea turtles; *D. coriacea*, *L. kempi* and *C. caretta* (Hooper and Ackman, 1970; Ackman *et al.*, 1971a; 1972) and two teleosts; the ocean sunfish, *Mola mola* (Hooper *et al.*, 1973; Ackman *et al.*, 1973) and the spadefish, *Chaetodipterus faber* (Pearce and Stillway, 1976.)

Since jellyfishes comprise the major portion of the diet of the leatherback turtle and at least a part of the diet of the Kemp's ridley and loggerhead (Pritchard, 1979; Mortimer, 1982), it seems probable that jellyfishes are the source of *trans* 16:1 ω 10 in fats of the carnivorous turtles. Of all the samples analyzed in this study, only the Hawaiian turtle fat contained *trans* 16:1 ω 10 in any significant amount. Chromatography based upon AgNO₃/TLC and analysis of fractions on polar and non-polar GLC columns indicated a component comprising 0.47% of the total to be *trans* 16:1 ω 10. Although the specific position of the ethylenic bond was not established by rigorous structural analysis, the chromatographic behavior of the component was consistent with that shown previously for *trans* 16:1 ω 10 (Hooper and Ackman, 1970).

Lower percentages of 18:2 ω 6 and 18:3 ω 3 and somewhat higher percentages of 16 and 20 carbon polyenes in the Hawaiian fat, compared to the Caribbean oil, suggest the inclusion of algae in the diet of the Hawaiian turtle although the 20 carbon polyenes could also be a contribution from dietary jellyfishes. While green turtles of the Caribbean retained less than 2% of their principal dietary fatty acids, 18:2 ω 6 and 18:3 ω 3, the presence of modest amounts of dietary 20 and 22 carbon polyenes and *trans* 16:1 ω 10 in the Hawaiian fat may indicate that the green turtle is less able to catabolize these fatty acids. Indeed, it has been suggested previously that *trans* 16:1 ω 10 is metabolically inert and therefore accumulates in the depot fats of the predator (Ackman *et al.*, 1971a, 1972; Hooper and Ackman, 1970). Furthermore, Lawson and Holman (1981) have reported a significantly reduced rate of β -oxidation of 18 carbon monoenes containing a Δ 4 or Δ 5 ethylenic bond, as compared with the Δ 9 monoene, in rat liver mitochondria. Consequently, the presence of an initial Δ 4 (22:6 ω 3) or Δ 5 (20:4 ω 6 and 20:5 ω 3) ethylenic bond may depress metabolism of long-chained polyenes by the green turtle.

The composition of the Hawaiian fat sample was similar to that of the Indian Ocean and Caribbean

oils in the proportions of the saturated fatty acids and their totals and also in the details of the isomeric monoethylenic fatty acids and their totals (Table 3). Although this fat sample had ten times as much 22:1 as the rendered oils, it too contained erucic acid (22:1 ω 9) as the dominant 22:1 isomer, rather than cetoleic acid (22:1 ω 11), the characteristic 22:1 isomer of fish oils including that of clupeids from northern latitudes (Ackman *et al.*, 1974b, 1980; Pascal and Ackman, 1976). This detail is well illustrated by the data of Table 2, comparing the fatty acid composition of the river herring lipids with that of the aquarium-reared albino green turtle depot fat. The principal 22:1 isomer in the aquarium-reared turtle fats was, as in the herring lipids, 22:1 ω 11 + 13. While this was an uncontrolled, but fortuitous, contribution to this study, the point made is an important one in marine food web studies utilizing fatty acids as marker compounds. Most marine animals not ingesting copepods, or primary copepod predators, contain very little 22:1 and that, mostly in the form of 22:1 ω 9 (Ackman, 1982).

The fatty acid composition of pen- and aquarium-reared green turtles reflected that of their diets to a remarkable degree. Unlike wild turtles, these turtles received an ample and constant food supply and were relatively restricted in their movement, conditions conducive to deposition of excess dietary fat. Even more remarkable than the obvious similarities in proportion of major components in the aquarium-reared turtle depot fat and herring lipids, was the presence of 4,8,12-trimethyltridecanoic acid (TMTD) in the turtle fat. This multi-methyl-branched isoprenoid fatty acid is a product of chlorophyll metabolism and common in lipids of invertebrate herbivorous grazers (Ackman *et al.*, 1971b; Johns *et al.*, 1980) and filter-feeders (Ackman *et al.*, 1970; 1974a). It is also found in the fats of marine mammals and fish, such as clupeids, that prey on filter-feeding zooplankton (Ackman and Hooper, 1968; Ackman and Eaton, 1966, 1971). Although TMTD can be produced by microbial action in ruminants (Patton and Benson, 1966; Hansen, 1966), it is clear that the microbial flora of the green turtle lacks this capability since TMTD was not detected in any of the pen-reared or wild turtle oils. Thus, TMTD in the aquarium-reared turtle fat reflects deposition of dietary fatty acids.

Arachidonic acid (20:4 ω 6) was present at about 1% in all of the turtle fats and oils and all samples contained 22:4 ω 6 and 22:5 ω 6, probably derived from 20:4 ω 6 by chain extension. This molecular elongation and desaturation is a process more often thought of as occurring in cellular membranes or organelles of vital organs where a high degree of polyunsaturated fatty acid specificity may be required. The need for such specificity in a depot fat which serves, in part, as a repository for unutilized dietary fat is unlikely. Thus, it appears that dietary 20:4 ω 6 and 20:5 ω 3, in parallel, are chain elongated and desaturated to 22:4 ω 6, 22:5 ω 6, 22:5 ω 3 and 22:6 ω 3 prior to deposition in the fats of the green turtle.

Regardless of diet, all of the green turtle fats and oils examined in this study contained substantial amounts of 12:0 and 14:0, probably biosynthesized

from acetate units derived from cellulose digestion and β -oxidation of fatty acids. Thus, these fatty acids are species-specific characteristics of green turtle oils.

CONCLUSIONS

Although the wild green turtle readily accepts animal matter in its diet, our evidence indicates that it utilizes a herbivorous diet more efficiently. Not only does it digest dietary cellulose (Bjorndal, 1979, 1980, 1982), but it also metabolizes dietary fatty acids and biosynthesizes fatty acids characteristic of the species. However, when animal matter is included in the diet, it appears that the green turtle is unable to readily metabolize the polyunsaturated fatty acids and therefore, they accumulate in the depot fat along with the species-specific fatty acids. In contrast, captive green turtles accumulate fatty acids of all dietary sources because they have a constant, ample food supply and restricted activity, as compared with wild turtles.

Acknowledgements—Samples of turtle depot fats or oils and diets were provided by: J. Wood, Grand Cayman Farm, Ltd., Grand Cayman Island; J. Lyons Co., London; Sea Farms, Inc., Key West FL; Esperis, S.P.A., Milan, Italy; A. Meylan, University of Florida, Gainesville, FL; and G. H. Balazs, Hawaii Institute of Marine Biology, Kaneohe, HI. Seagrasses were collected by B. Palko, T. Dean and G. Simms, National Marine Fisheries Service, Southeast Fisheries Center's Panama City Laboratory, Panama City, FL. Laboratory and technical assistance were provided by S. N. Hooper, C. A. Eaton, A. Timmins (Halifax Laboratory) and F. Van Dolah (Charleston Laboratory). The Canadian Wildlife Service provided support for Halifax facilities.

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