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Lipid composition of normal human bone marrow as determined by column chromatography*

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SUMMARY

The composition of the lipids of normal human bone marrow has been determined. Marrows were obtained post-mortem from the femurs of 12 subjects ranging in age from 1 to 78 years. The total extractable lipid, which varied from 28% to 84% of the tissue wet weight, was composed primarily of neutral lipid. It contained less than 3% of phospholipid and no detectable carbohydrate. The neutral lipid consisted of 96% to 98% triglyceride and minor amounts of free cholesterol and free fatty acids. Fractions representative of hydrocarbons, cholesterol esters, diglycerides, and monoglycerides were not detected. The fatty acids of the triglyceride fraction were analyzed by gas-liquid chromatography. The predominant fatty acids were palmitic and oleic, which accounted for an average of 26% and 46%, respectively, of the total. Myristic, palmitoleic, stearic, and linoleic acids were also present but in smaller amounts.

The composition of the lipids of bone marrow has been studied in the rabbit (1, 2), and the total amount of lipid has been determined for a number of species (3), but information as to the nature of these lipids in man is lacking. This investigation has been carried out with the express purpose of ascertaining the normal composition of the lipids of human bone marrow, a tissue of particular interest to us because of its relative importance in xanthomatous diseases.

METHODS AND MATERIALS

Tissues. Samples of marrow were obtained from the upper two-thirds of the femur. The average interval between death and removal of the specimen was 6 to 8 hours and during this period the bodies were refrigerated. Samples were freed of small bone fragments, weighed, sealed in aluminum foil, and immediately stored at −20° in the dark. Specimens were selected to cover the first through the seventh decade without regard to sex. Each marrow was examined microscopically and those with an abnormal histology were discarded. Patients were chosen who had no demonstrable lipid or hematopoietic disease and exhibited normal blood findings.

Solvents. With the exception of the *n*-hexane (Skellysolve B), all solvents were reagent grade. Benzene, ethyl ether, and *n*-hexane were distilled in glass before use. The anhydrous ethyl ether was dried over sodium before distillation and stored in the dark in a refrigerator. The composition of the solvent mixtures is expressed in volume per cent.

Lipid Extraction. The tissue extraction and washing of the extract were performed by the method of Folch *et al.* (4). The washed lipid extracts were dried in a rotary vacuum evaporator at not more than 40° and dissolved in solvent for storage and analysis.

Silicic Acid Chromatography. The conditions used were those of Barron and Hanahan (5) and Hirsch and Ahrens (6). Generally, 600 to 700 mg of lipid mixture was applied to a column containing 40 g of silicic acid and 10 g of Hyflo Super-Cel®. Elution was performed by successive use of the following solvent mixtures: 15% benzene-hexane and 5%, 20%, 30%, and 70% ethyl ether-hexane. Elution of cholesterol with 15% ether-hexane was incomplete; 20% ether-hexane, on the other hand, gave reproducible and complete elution.

Gas-Liquid Chromatography (GLC) of Fatty Acids. The triglycerides were saponified, and the isolated fatty acids were converted to their methyl esters according to the procedure of Radin *et al.* (7) and analyzed by GLC using the procedure of Lipsky *et al.* (8). A Beckman GC-2A instrument was used with a thermal conductivity detector and the 12-ft × 1/4-in. stainless

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TABLE 1. LIPIDS OF NORMAL HUMAN BONE MARROW

Age and Sex	Total Extractable Lipid	Composition of Total Lipids: Triglyceride*	Free Cholesterol	Phospholipid P	Red (Blood Forming) Marrow/Yellow (Fatty) Marrow†
	% *wet wt*	%	%	*μEq/g*	
1-F	44	97	0.5	1.5	90/10
12-M	28	96	0.9	0.1	70/30
16-M	57	97	0.4	0.2	50/50
23-F	58	96		1.1	50/50
28-M	63	97	0.2	0.0	40/60
39-M	60	98	0.4	0.6	40/60
51-M	64	97		0.2	40/60
59-M	43	97		1.6	40/60
61-M	55	96	0.4	0.3	40/60
62-F	61	96	0.3	0.3	40/60
72-M	65	98	0.3	0.0	40/60
78-M	84	96	0.3	0.0	20/80

* Free fatty acids are included in this fraction. Their contribution to the total was always less than 1%.

† Histological estimation.

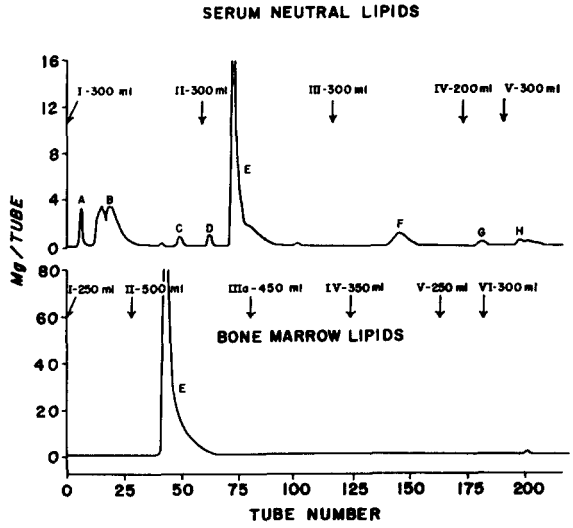

FIG. 1. Chromatographic patterns of the lipids of human bone marrow and the neutral lipids of human serum. Solvent changes and volumes are indicated by arrows. The solvent systems used were: I—15% benzene-hexane; II—5% ether-hexane; III—20% ether-hexane; IIIa—15% ether-hexane; IV—30% ether-hexane; V—70% ether-hexane; VI—methanol. The fractions eluted were as follows: A—"hydrocarbons"; B, C, and D—cholesterol or sterol esters; E—triglycerides and fatty acids; F—free cholesterol; G and H—other glycerides. For the bone marrow analysis, 515 mg of lipid was fractionated on a column containing 30 g of silicic acid; in the case of the serum lipids, 168 mg was separated using 13 g of silicic acid. The volume of solvent collected per tube was increased in the latter half of each fractionation.

steel column was loaded with diethylene glycol succinate (5.25 g) on C-22 firebrick (35.45 g). Gas pressure was maintained at 25 lb of helium, the filament current was 200 ma, and the column temperature was 220°. Under these experimental conditions, retention times for the methyl esters of palmitic, palmitoleic, stearic, and oleic acids were 19.0, 21.0, 33.3, and 35.7 minutes, respectively. Percentage composition of unknowns was calculated from the integrated areas under the eluted peaks. Deviations from theory were less than 2% when known mixtures were analyzed in this manner.

Analytical Methods. Total lipid in extracts and pooled fractions was determined by measuring the weight of the residue after evaporation of an aliquot at 100° to 110°. When the amount of specimen was limited, the dichromate method of Kibrick and Skupp (9) was employed using recrystallized palmitic acid as a standard. Total phosphorus was estimated by the method of King (10) and cholesterol by the method of Zak *et al.* (11). Free fatty acids were determined by titration in hexane with 0.02 N methanolic NaOH using thymol blue as an indicator (12, 13).

RESULTS

Data obtained by the analysis of 12 samples of human marrow by means of column chromatography are presented in Table 1. A typical analysis for one specimen is reproduced in Figure 1, along with the type of pattern obtained when human-serum neutral lipids are fractionated.[1] The latter is presented to demonstrate the degree of resolution of complex mixtures of neutral lipids possible under our experimental conditions. The total extractable lipid in the marrow specimens varied between 28% and 84% of the fresh tissue weight. It did not appear to be a function of age as has been suggested by others (14). In every instance, the triglyceride content of the marrow lipid was quite high and constant at 96% to 98%.

With respect to other lipid fractions, only free cholesterol and phospholipid were present in significant amounts. Esterified cholesterol, hydrocarbons, other neutral glyceride derivatives, and carbohydrate-containing lipids were not detected. The amount of phospholipid present varied but did not exceed 3% of the weight of the total extractable lipid. Further fractionation was precluded by the minor amounts obtained. Although free cholesterol did not exceed 1%

[1] Analysis by GLC has demonstrated that the bimodal peak B represents partial separation and the small peaks C and D reflect complete separation of sterol ester fractions that differ in their fatty acid components.

TABLE 2. PERCENTAGE FATTY ACID COMPOSITION OF THE TRIGLYCERIDE FRACTION

Age and Sex	Gas-Liquid Chromatographic Fatty Acid Distribution*						
	12:0	14:0	16:0	16:1	18:0	18:1	18:2
1-F	1.1	4.1	28.7	4.1	11.3	43.1	6.8
12-M	0.9	3.8	21.6	5.0	11.2	44.6	11.0
16-M	0.2	2.9	25.9	5.1	6.8	47.2	11.3
23-F	0.5	2.8	23.3	4.6	10.6	46.3	11.1
28-M	0.3	3.8	25.9	8.2	4.5	47.0	8.2
39-M	0.4	3.3	31.7	3.1	9.8	42.7	8.8
51-M	0.7	3.3	28.4	3.5	8.9	45.9	9.2
59-M	0.2	3.0	31.7	4.9	5.8	46.2	7.4
61-M	0.3	2.3	22.5	10.8	6.2	48.5	7.9
62-F	0.2	2.7	25.2	11.5	6.2	47.1	6.0
72-M	0.4	3.7	23.3	7.5	10.9	46.9	5.6
78-M	0.4	3.0	27.4	7.9	3.6	51.7	3.9

* Trace fractions were observed but are not recorded since their structure is uncertain. They were, however, included when the percentage fatty acid distribution was calculated.

of the total lipid weight, its presence is regarded as significant, and not a result of contamination of the specimen with blood since cholesterol esters were absent.

Our results indicate that the lipids of normal human marrow are very constant in composition, consisting of virtually pure triglyceride. It was, therefore, interesting to determine the distribution of fatty acids in this fraction by means of GLC (Table 2). The fatty acids present ranged from C_{12} to C_{18} in chain length. Although a specific search was made for arachidonic acid because of its important role in human nutrition, significant amounts could not be detected. Two acids, palmitic and oleic, accounted for approximately 75% of the total. Lesser but significant amounts of lauric, myristic, palmitoleic, stearic, and linoleic acids were also present.

DISCUSSION

The lipid composition of human marrow is similar to that of the rabbit (2), in which triglycerides predominate and cholesterol and phospholipids are minor constituents. It differs from that of compact bone of the ox (15) in that the extractable lipid of the latter contains as much as 13% cholesterol and 1.7% cholesterol esters. The levels of phospholipid and cholesterol in the lipids of bone marrow may have some relation to the hematopoietic cellularity of the tissue (Fig. 2). It is of considerable interest that the triglyceride fraction accounts for 96% to 98% of the extractable lipid in all specimens despite considerable variations in the age of the donor, total lipid content, and hematopoietic cellularity. The distribution of fatty acids in marrow triglycerides was also consistent and in general agreement with the proportions of saturated and unsaturated fatty acids reported for human fat by Mattson and Lutton (16). The distribution of fatty acids in human plasma triglycerides (17) is also comparable to that of the marrow triglycerides, except for the greater proportion of C_{18} and C_{20} polyunsaturated acids in the former. There appears, therefore, to be little analytical basis for a differentiation between the lipids of bone marrow and adipose tissue, a finding that is in accord with the observation of Evans *et al.* (18) who concluded that marrow fats represent predominantly a form of lipid storage.

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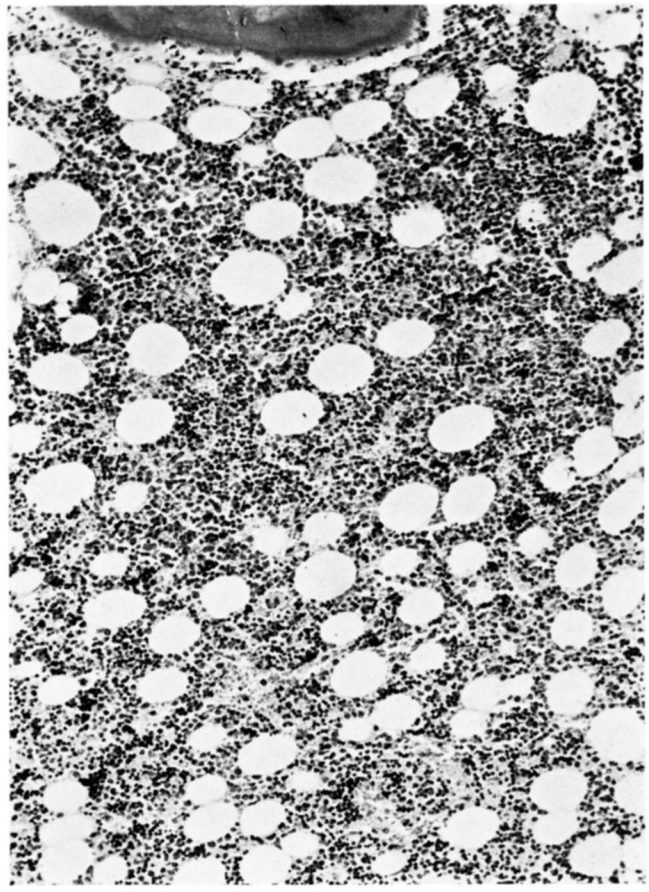

FIG. 2. Photomicrograph of a histological preparation of femoral bone marrow from a 63-year-old male (× 110). The fat cells appear as vacuoles.

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