# Lipids of human leukocytes: relation to cell type

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ABSTRACT Significant differences in lipid composition have been found between normal human lymphocytes and polymorphonuclear leukocytes (isolated from blood by means of glass-bead columns), abnormal leukocytes from patients with acute and chronic leukemia, and leukocytes from peritoneal exudates. Lipid extracts of isolated leukocytes were analyzed for total lipid, phosphorus, cholesterol, and plasmalogens. Individual phospholipids and neutral lipids were separated by thin-layer chromatography.

The major phospholipids were phosphatidyl choline, ethanolamine glycerophosphatides, sphingomyelin, phosphatidyl serine, and phosphatidyl inositol. Plasmalogen was found mainly as phosphatidal ethanolamine. The neutral lipid fractions contained free cholesterol and various amounts of triglyceride, but little esterified cholesterol. Normal lymphocytes contained about half as much total lipid per cell as normal polymorphonuclear leukocytes, with a similar cholesterol:-lipid-P ratio but relatively more lecithin and less ethanolamine glycerophosphatide. Normal mature leukocytes, compared with immature cells of the same morphological series, had a higher total lipid content per cell, more cholesterol, and a higher ratio of cholesterol to lipid-P. Little difference was found in total lipid-P per cell, but mature cells contained relatively less lecithin and more sphingomyelin.

These findings may reflect differences in the relative content of various intracellular organelles as well as possible differences in the quantity and composition of the plasma membrane.

KEY WORDS man · lymphocyte · polymorphonuclear leukocyte · glass-bead · separation · leukemia · erythrocyte · thin-layer chromatography · phospholipid · cholesterol · plasmalogen · neutral lipid

HUMAN LEUKOCYTES have received relatively little attention from lipid chemists. The few reports dealing with the lipids of human leukocytes are largely based on studies of mixed leukocyte populations ("buffy coat" preparations) (1–6) or of abnormal cells such as leukemic leukocytes (4, 7–12). Various types of mammalian cells have been used as models of particular leukocyte types, such as phagocytic cells harvested from peritoneal exudates (13–15) or lymphocytes from thoracic duct lymph (16). It is known that erythrocytes of different mammalian species may differ widely in lipid composition and metabolism (17), but virtually no information is available about differences in these properties between the normal human leukocytes of circulating blood and those derived from disease states, sources other than blood, or animals.

For the present investigation, normal human lymphocytes and polymorphonuclear leukocytes were isolated from blood in relatively pure form by the glass-bead column method of Rabinowitz (18). Comparison of the lipid composition of these cells with that of abnormal leukocytes of several types showed a characteristic pattern of lipid content for each cell type, with some significant differences between normal and abnormal cells of a given morphological series.

## MATERIALS AND METHODS

### Sources of Blood

Venous blood in volumes up to 250 ml was obtained immediately before use from normal volunteers. The specimens were collected by gravity flow through plastic tubing into plastic or siliconized glass containers. For each 100 ml of blood, 1 ml of heparin solution (1000 units per ml in 0.15 M NaCl) was added as an anticoagulant. For experiments not requiring fractionation of the leukocyte suspension into individual cell types, heparin was replaced by a 10% solution of disodium EDTA. Blood samples containing abnormal leukocytes were obtained from patients hospitalized at the Bronx Municipal Hospital Center.

### Glassware

All glassware, beads, and polycarbonate vessels used in the collection and handling of the intact cells were siliconized with "Siliclad" (Clay-Adams, Inc., New York). Plastic vessels, other than polycarbonate tubes, did not require silicone treatment.

### Sedimentation of Erythrocytes with Dextran

To each 100 ml of anticoagulated blood, 6 ml of 5% glucose and 10 ml of 6% dextran (molecular weight about 225,000) in 0.15 M NaCl were added and thoroughly mixed by inversion. The suspension was transferred to a tall polyethylene or siliconized glass cylinder and placed in a water bath at 37°C. After about 15 min, when the suspension had separated into two well-defined layers, the upper layer (containing mostly leukocytes and platelets) was stirred gently with a glass rod to dislodge erythrocytes adhering to the sides of the cylinder. Sedimentation of erythrocytes was then continued for an additional 30 min. The upper phase—a dark yellow, turbid suspension—contained most of the leukocytes and platelets. When the initial leukocyte count was normal (5,000–10,000 per mm³), the suspension usually contained 100–300 erythrocytes per 100 leukocytes at this stage. The mixture was transferred to 50-ml tubes and centrifuged in an International model HN centrifuge for 10 min at 200 g. Centrifuge speed and timing were critical: too much centrifugal force tended to cause irreversible clumping, whereas too little resulted in loss of cells, especially the lighter lymphocytes. The leukocyte button was resuspended in about 5 ml of plasma and transferred to a column of glass beads for separation of the lymphocyte and polymorphonuclear leukocyte fractions. For studies of mixed leukocyte populations or of specimens in which a single abnormal cell type predominated, the column separation step was omitted.

### Separation of Lymphocytes and Polymorphonuclear Leukocytes

The glass-bead column method of Rabinowitz (18) was employed for the separation of normal lymphocytes from polymorphonuclear leukocytes. For 250 ml of normal blood (approximately 2 × 10⁹ cells), a water-jacketed column of 25 mm I.D. was filled with siliconized glass beads, 0.2 mm in diameter (Minnesota Mining and Mfg. Co., St. Paul, Minn.), to a height of 70 mm. The leukocyte suspension was retained on the column for 30 min at 37°C before elution with 85 ml of undiluted fresh plasma followed by 150 ml of 20% plasma in Hanks' balanced salt solution for lymphocytes, and 350 ml of a salt solution containing EDTA for polymorphonuclear leukocytes (18). Constant flow rates of 2.7–5.2 ml/min were maintained with a Harvard syringe pump. Effluent leukocyte suspensions were collected directly into siliconized test

tubes in a cold water bath. Each tube was provided with a micro spin bar, and clumping was minimized by gentle agitation of the suspensions with a magnetic stirrer.

### Removal of Erythrocytes by Hypotonic Lysis

Most of the erythrocytes still present in the leukocyte suspension after sedimentation with dextran emerged from the glass-bead column together with lymphocytes in the first few tubes. These residual erythrocytes were removed by brief exposure to hypotonic saline (19), a procedure which produced lysis of the erythrocytes without significantly damaging the leukocytes. Erythrocyte ghosts and hemoglobin were removed in the subsequent washing procedure.

### Washing of Isolated Leukocytes

Leukocyte suspensions were washed 3–4 times with Hanks' medium and the cells were collected after each washing by centrifugation at 200 g for 10 min. The addition of 10% disodium EDTA solution (about 0.05 ml/10 ml of leukocyte suspension) just before centrifugation seemed to improve yields by inhibiting clumping. Between operations, the leukocyte suspensions were agitated in a cold water bath with the aid of a magnetic stirrer.

### Evaluation of Purified Leukocyte Preparations

Cell counts were performed in duplicate or quadruplicate with a Spencer "Bright-Line" hemacytometer (American Optical Company, Buffalo, N.Y.). Cell morphology, motility, and exclusion of trypan blue (19) or eosin (20) were evaluated by examination of wet coverslip preparations by phase microscopy. Dry smears were prepared by mixing a drop of cell suspension with a drop of autologous plasma, spreading the mixture over the slide with a glass rod, and allowing the smear to dry slowly in a humid atmosphere. The smears were fixed with methanol for a few minutes and then stained with Wright's stain.

### Extraction of Lipids

Lipids were extracted from cell suspensions immediately after the final washing. All organic solvents were redistilled, reagent grade solvents. Extracting solvents were deaerated with nitrogen just before use, and extractions were performed with cold solvents at 2–5°C. 1 volume of cell suspension was added to 7 volumes of methanol and the mixture was stirred constantly with a magnetic stirrer for 15 min; 14 volumes of chloroform were then added, and stirring was continued for 1 hr. The mixture was filtered through solvent-washed filter paper, and the residue was then subjected to a second extraction with 10 volumes of chloroform–methanol 2:1. No significant increase in yield of lipid phosphorus was obtained by prolonging the extraction time beyond 1 hr or by increasing the temperature of the solvents to room temperature or

higher. The chloroform–methanol extracts were washed by the method of Folch, Lees, and Sloane Stanley (21), with 0.1 M KCl in the upper phase. The lower phase was taken to dryness in a rotary evaporator at a temperature no higher than 37°C, and the lipid was redissolved immediately in cold chloroform–methanol 2:1. The crude lipid extracts were stored at −20°C in tightly stoppered amber bottles.

## Analytical Methods

Lipid phosphorus was determined by the method of Beveridge and Johnson (22), with the addition of sodium sulfite as recommended by Morrison (23) to neutralize any residual hydrogen peroxide at the end of digestion. For determination of small quantities (0.2–2.0 $\mu$g) of phosphorus, the blue complex was extracted with a small volume of $n$-butanol [an adaptation from the method of Berenblum and Chain (24) suggested by Dr. M. M. Rapport[1]]. In $n$-butanol extracts, the absorption maximum shifted from 830 m$\mu$ to 795 m$\mu$. Spectrophotometry was performed with a Zeiss PMQ II spectrophotometer. Cholesterol was determined by the ferric chloride procedure of Leffler (25). Plasmalogens were estimated as $\alpha,\beta$-unsaturated ethers by the spectrophotometric iodination method of Gottfried and Rapport (26), with the +8% correction factor recommended by Rapport and Norton (27). For determination of total lipid weight, aliquots of the washed extracts were evaporated to dryness in previously weighed vessels under a stream of nitrogen at 37°C. The samples were dried overnight in a vacuum desiccator over phosphorus pentoxide and then weighed on a Mettler H16 balance or a Cahn "Gram" Electrobalance.

## Thin-Layer Chromatography

"Basic" chromatoplates of 0.25 mm nominal thickness were prepared by the method of Skipski, Peterson, and Barclay (28) from slurries of Merck Silica Gel H (30 g) and 0.001 M Na$_2$CO$_3$ (80 ml). In a few experiments, Silica Gel H washed by the method of Parker and Peterson (29) or Silica Gel HR (Merck) was substituted for unmodified Silica Gel H, but separations and recovery were not notably improved. The plates were activated for 30 min at 110°C just before use. A developing solvent consisting of chloroform–methanol–glacial acetic acid–water 25:15:4:2 (28) produced the best separations. Despite the presence of acetic acid in the developing solvent, no detectable breakdown of plasmalogens occurred during chromatography. Lipid extracts were also studied by chromatography on "neutral" Silica Gel G (or H) with chloroform–methanol–concentrated ammonia–water 70:-30:2:3 or with diisobutyl ketone–acetic acid–water 40:25:5 (30). Neutral lipids were separated by chroma-

tography on Silica Gel H with hexane–diethyl ether–acetic acid 80:20:1.

Standard lipids were obtained from Applied Science Laboratories, Inc., State College, Pa., and the Sylvana Chemical Company, Millburn, N.J. A phosphatidyl inositol standard of soybean origin was kindly donated by Dr. Guiseppe Colacicco, and Dr. Vladimir Skipski generously supplied a sample of phosphatidyl inositol prepared from wheat sprouts by Dr. M. Faure. Lipid spots were detected by exposure to iodine vapor or by charring with sulfuric acid. Free amino groups were identified with ninhydrin spray reagent (0.5% ninhydrin in $n$-butanol saturated with 0.05 M sodium acetate). Phosphorus-containing lipids were localized with the reagent of Dittmer and Lester (31). Plasmalogens were detected with the Schiff reagent (32).

The identity of individual lipid spots was confirmed in some instances by IR spectrophotometry after they had been eluted from the silica gel plates. A Perkin-Elmer model 237B spectrophotometer equipped with a 4× beam condenser was employed for the examination of lipid samples of 80–100 $\mu$g. Specimens were examined as thin films on NaCl windows or as solutions in chloroform or carbon tetrachloride.

## Quantitative Thin-Layer Chromatography

Chromatoplates (5 × 20 cm) prepared with "basic" Silica Gel H were marked off into three lanes about 1.5 cm in width. Duplicate samples containing about 5–6 $\mu$g of lipid phosphorus were applied to the outer lanes; the center lane was left blank. The chromatogram was then developed for about 40 min in chloroform–methanol–acetic acid–water 25:15:4:2 in a rectangular tank (Desaga) lined with Whatman No. 1 filter paper. The developed chromatograms were allowed to dry in air for a few minutes and were then exposed to iodine vapor until the lipid spots appeared. The area corresponding to each lipid spot was marked off, along with equivalent "blank" areas of silica gel. Each area thus marked off was scraped from the plate with a sharp stainless steel spatula and transferred directly to a 25 × 200 mm digestion tube for phosphorus analysis. With the phosphorus method described ($n$-butanol being used for color extraction) the presence of silica gel in the reaction mixture did not affect color development or spectrophotometric readings. Silica Gel H gave blank values corresponding to less than 0.05 $\mu$g of phosphorus per cm$^2$. Recovery of phosphorus from the chromatograms averaged 94% of the total lipid phosphorus in the starting samples.

With chloroform–methanol–acetic acid–water 25:15:-4:2 v/v and "basic" Silica Gel H, phosphatidyl serine could not be separated completely from phosphatidyl inositol. The relative proportions of these two phosphatides were determined by separation of some of the

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[1] Personal communication.

TABLE 1   LIPIDS OF HUMAN LEUKOCYTES AND ERYTHROCYTES

| Cell Type/Source | No. of Patients | Total Lipid | Lipid Phosphorus | Cholesterol | Cholesterol: Lipid-P | α,β-Unsaturated Ether: Lipid-P |
|---|---|---|---|---|---|---|
| | | $g \times 10^{-12}/cell$ ± SD | $g\ atoms \times 10^{-15}/cell$ ± SD | $moles \times 10^{-15}/cell$ ± SD | molar ratio | molar ratio |
| Normal leukocytes, mixed population | 4 | 15.55 ± 4.5 | 9.01 ± 1.69 | 5.21 ± 1.06 | 0.58 | 0.25 |
| Lymphocytes, normal* | 5 | 11.1† | 4.73 ± 0.93 | 3.24 ± 0.30 | 0.68 | 0.14 |
| Lymphocytes, chronic lymphocytic leukemia‡ | 6 | 5.44 ± 0.91 | 4.00 ± 0.53 | 1.54 ± 0.19 | 0.38 | 0.15 |
| Lymphoblasts, acute lymphocytic leukemia§ | 1 | 5.96 | 4.28 | 1.08 | 0.25 | 0.19 |
| Polymorphonuclear leukocytes, normal* | 6 | 21.54 ± 1.16 | 9.67 ± 1.11 | 5.33 ± 0.70 | 0.52 | 0.24 |
| Polymorphonuclear leukocytes, peritoneal exudate‖ | 1 | 18.3 | 11.9 | 7.92 | 0.67 | 0.22 |
| Myeloid metaplasia leukocytes¶ | 1 | 16.8 | 10.4 | 5.26 | 0.51 | 0.22 |
| Chronic myelogenous leukemia** | 2 | 13.4 | 10.2 | 4.98 | 0.49 | 0.24 |
| Acute myelogenous leukemia†† | 1 | 13.3 | 8.13 | 3.15 | 0.39 | 0.26 |
| Monoblasts, acute monocytic leukemia‡‡ | 1 | 19.6 | 19.0 | 5.92 | 0.31 | 0.20 |
| Erythrocytes, normal§§ | 7 | 0.457 ± 0.025 | 0.354 ± 0.010 | 0.341 ± 0.015 | 0.96 | 0.17 |

* Separated from blood by glass-bead columns (see text).
† Tentative value; quantities insufficient for precise determination.
‡ Small lymphocytes 95–100%.
§ Lymphoblasts 98%.
‖ Polymorphonuclear leukocytes 95%, mononuclear cells 5%, (from a patient with a liver tumor).
¶ Mature polymorphonuclear leukocytes 80%, immature granulocytes 15%.
** Mature polymorphonuclear leukocytes 50–56%, immature myeloid cells 40–42%.
†† Myeloblasts 30%, immature and mature granulocytes 63%.
‡‡ Monocytic cells 67%, promyelocytes 21%.
§§ Calculated on the basis of $1.15 \times 10^{10}$ erythrocytes per ml of packed cells, mean corpuscular volume 87 $\mu^3$.

extracts by means of a two-stage chromatographic procedure. A preparative thin-layer chromatogram was first developed in chloroform–methanol–acetic acid–water 25:15:4:2 containing 0.05% 2,6-di-*tert*-butyl-*p*-cresol as an antioxidant (33). The spot corresponding to phosphatidyl serine plus phosphatidyl inositol was identified under UV light after the plate had been lightly sprayed with 0.001% 2′,7′-dichlorofluorescein in ethanol. The lipid was eluted from the silica gel with chloroform and methanol, and was then concentrated and applied to a second Silica Gel H chromatoplate for development with diisobutyl ketone–acetic acid–water 40:25:5. The second chromatographic system, in the absence of lecithin and sphingomyelin, separated phosphatidyl serine cleanly from phosphatidyl inositol.

## RESULTS

Normal lymphocytes and polymorphonuclear leukocytes were separated into relatively pure fractions by the glass-bead columns, with yields averaging 19% of the initial cell count. In each fraction, cells of the specified type averaged 98% of the total leukocyte count. Viability, as determined by trypan blue exclusion, averaged 95% in the final cell suspension. Contamination with erythrocyte ghosts and platelets averaged 4 and 10 per 100 leukocytes, respectively, when initial leukocyte counts were normal. In comparison with leukocytes, erythrocytes and

platelets contain very little lipid per cell; therefore, these cellular elements probably contributed less than 1–2% of the total lipid in the final extracts. In specimens with a high initial leukocyte count, such contamination of the final extracts was even smaller.

In addition to normal leukocytes, abnormal cells were obtained from six patients with chronic lymphocytic leukemia (lymphocytes), two with chronic myelogenous leukemia (mature and immature myeloid leukocytes), one each with acute myelogenous leukemia and acute lymphocytic leukemia (blast cells), and one with acute monoblastic leukemia (monoblasts). Additional specimens of polymorphonuclear leukocytes were obtained from blood of a patient with myeloid metaplasia and from ascitic fluid removed from a patient with a liver tumor. For comparison, lipid values of normal human erythrocytes are included in the tables.

Table 1 shows the total lipid content, lipid phosphorus, cholesterol, and plasmalogen content of each cell type. Total lipid content varied considerably among different cell types, roughly in proportion to cell size.

Normal polymorphonuclear leukocytes contained about twice as much lipid as lymphocytes and almost fifty times as much as erythrocytes. Phospholipid and cholesterol content of normal polymorphonuclear leukocytes and lymphocytes constituted about 35% and 10% of the total lipid, respectively, in contrast to the much higher proportion of each of these lipids in erythrocytes. The

cholesterol:phospholipid molar ratio, which approached 1.0 in erythrocytes, was only 0.52 in polymorphonuclear leukocytes and 0.68 in lymphocytes. Extracts of mixed populations of normal leukocytes gave figures that reflected the increased variability of lipid content in a mixture of cells, even though most of the lipid was derived from the polymorphonuclear leukocytes.

The ratio of $\alpha,\beta$-unsaturated ether to lipid phosphorus (Table 1) was considerably higher in polymorphonuclear leukocytes (0.24) than in lymphocytes (0.14). In thin-layer chromatograms sprayed with the Schiff reagent for identification of plasmalogens, only the ethanolamine glycerophosphatide spot gave a clearly positive reaction. This finding suggests that phosphatidal ethanolamine may constitute as much as 72% of the ethanolamine glycerophosphatide fraction in normal polymorphonuclear leukocytes and 49% in lymphocytes.

Abnormal leukocytes showed a pattern of decreased total lipid and decreased cholesterol in the immature cells of both the lymphocytic and the myeloid series, with relatively little alteration in the total lipid phosphorus content per cell. A striking decrease in the molar ratio of cholesterol to lipid phosphorus was apparent in the blast cells of the acute leukemias.

Examination of the neutral lipids by thin-layer chromatography demonstrated generally a large free cholesterol content, smaller amounts of triglyceride (which were different in different cell types), and very little esterified cholesterol. The predominance of free cholesterol was confirmed by chemical estimation of cholesterol in the lipid extracts. The digitonin-precipitable fraction contained 94% of the cholesterol in normal mixed leukocyte preparations, 94% in normal polymorphonuclear leukocytes, and 65% in normal lymphocytes.

Table 2 shows the phospholipid distribution in each of the cell types, as determined by quantitative thin-layer chromatography. Normal leukocytes, like erythrocytes, contained about 30% of the total phospholipid in the ethanolamine glycerophosphatide fraction (28.7% for lymphocytes, 33.4% for polymorphonuclear leukocytes). On the other hand, both lymphocytes and polymorphonuclear leukocytes contained more phosphatidyl choline (44% and 39% respectively compared with 29% for erythrocytes), and much less sphingomyelin (10% for both leukocyte types vs. 26% for erythrocytes). Comparison of normal and abnormal leukocytes suggested that, within each morphological series, increasing maturity was accompanied by an increase in the relative content of sphingomyelin and a decrease in phosphatidyl choline.

Thin-layer chromatography with chloroform–methanol–glacial acetic acid–water 25:15:4:2 did not reliably separate phosphatidyl serine from phosphatidyl inositol. These two components were therefore reported together in Table 2. In erythrocytes, this fraction was almost all phosphatidyl serine; however, in leukocyte extracts subjected to a two-stage chromatographic separation, about half of this fraction proved to be phosphatidyl inositol (39% in a normal mixed leukocyte population, 44% in

TABLE 2   PHOSPHOLIPID CONTENT OF HUMAN LEUKOCYTES AND ERYTHROCYTES

| Cell Type/Source* | No. of Patients | Phosphatidyl Choline | Ethanolamine Glycerophosphatides | Phosphatidyl Serine Plus Phosphatidyl Inositol | Sphingomyelin | Cardiolipin† | Other‡ |
|---|---|---|---|---|---|---|---|
| | | | | *mole percent composition* ± SD | | | |
| Normal leukocytes, mixed population | 4 | 39.4 ± 0.9 | 30.8 ± 2.4 | 14.2 ± 0.8 | 12.2 ± 0.9 | 2.5 ± 1.9 | 0.9 ± 0.6 |
| Normal lymphocytes | 8 | 43.6 ± 2.0 | 28.7 ± 1.8 | 13.4 ± 1.8 | 10.1 ± 2.6 | 3.2 ± 2.4 | 1.0 ± 1.1 |
| Lymphocytes, chronic lymphocytic leukemia | 6 | 49.5 ± 1.0 | 26.6 ± 2.0 | 13.2 ± 0.8 | 6.6 ± 0.9 | 3.9 ± 1.9 | 0.3 ± 0.5 |
| Lymphoblasts, acute lymphocytic leukemia | 1 | 50.6 | 26.0 | 14.2 | 4.0 | 5.0 | 0.2 |
| Normal polymorphonuclear leukocytes | 5 | 38.6 ± 1.8 | 33.4 ± 1.1 | 15.0 ± 1.1 | 10.5 ± 0.7 | 1.3 ± 0.3 | 1.1 ± 1.0 |
| Polymorphonuclear leukocytes, peritoneal exudate | 1 | 46.4 | 29.6 | 15.6 | 7.7 | 0.6 | 0.1 |
| Myeloid metaplasia | 1 | 39.7 | 30.9 | 10.6 | 15.1 | 3.4 | 0.3 |
| Chronic myelogenous leukemia | 2 | 39.9 | 31.3 | 14.4 | 10.2 | 2.2 | 2.0 |
| Acute myelogenous leukemia | 1 | 53.8 | 29.2 | 8.2 | 6.9 | 0.6 | 1.3 |
| Monoblasts, acute monocytic leukemia | 1 | 44.8 | 26.3 | 13.0 | 13.4 | 2.4 | 0.1 |
| Erythrocytes, normal | 8 | 29.2 ± 1.3 | 29.3 ± 0.9 | 14.2 ± 1.1 | 26.0 ± 1.0 | 0.9 ± 0.6 | 0.4 ± 0.1 |

* See Table 1 for detailed description.
† Includes phosphatidic acid and unidentified substances migrating near solvent front in thin-layer chromatograms.
‡ Includes lysolecithin and unidentified substances near origin in chromatograms.

polymorphonuclear leukocytes derived from peritoneal exudate, and 58% in lymphocytes from a patient with chronic lymphocytic leukemia).

## DISCUSSION

Numerous methods have been proposed for the separation of individual leukocyte types, based on such characteristic properties as differences in cell density (34–37), phagocytic ability (38–40), adhesiveness to glass surfaces (18, 41–43), accumulation in disease states (4, 7–12), or concentration in peritoneal exudates (13–15). The ideal separation system for normal cells, combining high yields and purity with minimal cell damage, is yet to be devised. In theory, a differential sedimentation (or centrifugation) method would offer the best possibility of avoiding cell damage during separation, but it is difficult to achieve good yields with acceptable purity by this means. In this laboratory, the best results were obtained by the glass-bead column method of Rabinowitz (18), which produced viable suspensions of both lymphocytes and polymorphonuclear leukocytes in moderate yield with a high degree of purity. Comparison with cells not subjected to this procedure gave no evidence of any differential lipid loss from either the lymphocyte or polymorphonuclear leukocyte fractions; indeed, these normal cells invariably showed a higher lipid content than leukemic leukocytes of the same morphological class.

Handling of the leukocyte preparations at all stages up to the actual extraction of lipids required special care if irreversible clumping and cell degeneration were to be avoided. The particular sensitivity of the ethanolamine glycerophosphatides to oxidative destruction is well known (44). When isolation and extraction procedures were carried out carefully with cold, deaerated extracting solvents, the ethanolamine glycerophosphatide fraction was never less than 25 moles per cent of the total phospholipid. Human erythrocytes, subjected to lipid extraction and quantitative phospholipid analysis by the method described above, gave values that agreed closely with those of Bradlow, Rubenstein, and Lee (45, 46), and Dodge and Phillips (44).

These studies have demonstrated not only significant differences in the lipid composition of normal human leukocytes of different morphological type, but also considerable differences between normal and leukemic leukocytes of the same apparent morphological series. Chemical differences between normal cells known to differ in origin, function, and morphology were not unexpected. The altered lipid composition in leukemic leukocytes merits further consideration. Reduction of cholesterol content in the cells of acute leukemia was the most prominent change observed. There was in addition a small but significant increase in the relative content of lecithin.

The reciprocal relationship between lecithin and sphingomyelin content is reminiscent of a similar relationship between these two choline-containing phospholipids in the erythrocyte membranes of different mammalian species (17).

The observed differences in leukocyte lipid composition may reflect differences in the relative content of various intracellular organelles, each with its own characteristic composition, as well as possible differences in the quantity and composition of the plasma membrane. Investigation of the lipids of subcellular fractions of these cells may help to clarify this point.

The variation found among the different kinds of leukocytes underscores the limited value of generalizations regarding lipid composition or metabolism derived from studies of mixed leukocyte populations or abnormal cells. Since even the comparatively simple cell membrane of the erythrocyte is known to vary extensively in lipid composition among different mammalian species (17), caution may be required in extrapolating the results of experiments based on animal leukocytes, until the relationship of these cells to normal circulating human leukocytes is established.

The possibility of obtaining pure samples of living cells of several different kinds from a single specimen of blood offers a research tool of particular usefulness for metabolic studies. This technique merits wider application in clinical investigation.

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