thors already quoted, Steelman *et al*(2) report a modification of the original Meier method(1) which could detect phenylbutazone at doses of 200-2000 mg/kg of diet without influencing body, thymus, or adrenal weight. Winder *et al*(4) have described Cl-440; flufenamic acid (N-(*a*,*a*,*a*-trifluoro-m-tolyl) anthranilic acid) as being 3.6 (1.2-40) (95%) times as active as phenylbutazone in a subcutaneous cotton pellet test in rats.

Winder *et al*(3) used a modified Meier cotton pellet antigranulation method in intact and adrenalectomized rats for assay of Cl-473 (N-(2,3-xylyl) anthranilic acid, mefenamic acid) in comparison to phenylbutazone. The anti-inflammatory effects were considered to be independent of the adrenal glands. The compound by the oral route was judged to be 0.6 (0.1-2.1) (95%) as potent as phenylbutazone. The difference between the slopes for cortisol and phenylbutazone was so large that relative potencies could not be determined.

Winter *et al*(5) have reported a new compound, indomethacin [1-(p-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid] which is an orally active inhibitor of cotton pellet granuloma formation in both the intact and adrenalectomized rat administered either systematically or locally. The compound has a relative potency 85 times phenylbutazone and about 4 times cortisol.

To this group we now add chloropheniramine maleate, dipyrone, aminopyrine, cinchophen and antipyrine as anti-inflammatory agents as judged by the cotton ball granuloma assay method.

Summary. An anti-inflammatory granuloma assay in the adrenalectomized rat has been employed to evaluate the relative potency of various nonsteroidal compounds. Statistically significant responses have been observed, but dose-related responses were not obtained. Graphic estimations of the range of relative potencies using cortisol acetate as the standard with an assigned potency of 100 follows: chloropheniramine maleate, 28-55; dipyrone, 3-8; phenylbutazone, 2-16; aminopyrine, 2-14; cinchophen, 2-18; and antipyrine, 1-5.

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Fatty Acid Composition of Sphingomyelin and Lecithin in Normal Human Serum.* (30321)

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The three major phospholipids in human blood serum are lecithin, lysolecithin and sphingomyelin. Of these, lecithin makes up approximately 67% of the serum phospholipids, while sphingomyelin and lysolecithin make up 21% and 8% respectively(1,2). Studies of the fatty acid composition of serum lecithin(3,4) indicate that it is comprised primarily of 16:0,† 18:0, 18:1 and 18:2 fatty acids. Human serum sphingomyelin, on the

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† The chain length of the fatty acid is given by the numeral before the colon and the number of double bonds by the numeral after it.

TABLE I. Serum Lipid Analysis.

Subject	Age	Sex	Total cholesterol, mg %*	Glyceride glycerol, mg/l	Total phospholipid, mg %	Lecithin plus lysolecithin, mg %	Sphingomyelin, mg %
1	16	♂	188	117	211	138	62
2	18	♀	224	106	214	173	48
3	20	♂	—	—	184	113	61
4	23	♀	190	—	272	196	62
5	29	♂	181	—	250	189	50
6	35	♂	—	—	205	158	40
7	41	♀	203	222	214	199	42
8	44	♀	242	133	282	238	54
9	49	♂	300	256	294	—	40
10	56	♀	310	146	336	—	46

* All values are less than the 90th percentile of a group of normals studied by Keys *et al* (15).

other hand, contains primarily saturated and monounsaturated fatty acids ranging from 14 through 25 carbon atoms, the major components being 16:0, 18:0, 22:0 24:1 and 24:0 (5). The variability of the fatty acid composition of these serum phospholipids in normal man is not known because previous assays have been performed on a limited number of individuals(3,4), on total serum phospholipids, on pooled serum specimens(5), or in different species(6). This report gives analyses of the fatty acids of serum lecithin and sphingomyelin in a series of 10 normal humans.

Materials and methods. Venous blood was drawn from 10 normal adult humans who were fasting but on unrestricted diets and who were not hospitalized. The subjects ranged in age from 16 to 56 years and included 5 males and 5 females. Six subjects were members of a monozygotic twin pair and 4 others were laboratory personnel. All had normal serum lipids (Table I).

Blood was allowed to clot and serum was separated by centrifugation and stored at —20°C. Serum cholesterol was measured by the method of Abell(7), serum phospholipids by the method of Chen, Toribara and Warner (8) and serum triglycerides by the method of Blankenhorn, Rouser and Weimer(9). For analysis of phospholipid fatty acids, 5 ml of serum was extracted with 20 volumes of chloroform-methanol 2:1 by homogenization in a Waring blendor. The extract was filtered on a sintered glass filter, the protein cake was reextracted with 10 volumes of chloroform-methanol 2:1 and the extract refiltered. The combined filtrates were evaporated to dryness on a rotary flash evaporator and the total extract was weighed to estimate total serum lipid. The total lipid extract was then fractionated on a column of acid silicic acid prepared as described previously(10). The column height was 15 cm and the i.d. 2.5 cm. The column was prewashed with chloroform and the total lipid extract was applied to the column in chloroform-methanol 4:1 (250 ml). Two fractions were collected, the first was eluted with chloroform-methanol 4:1 (250 ml), the second with methanol (150 ml). The chloroform-methanol 4:1 eluate contained triglycerides, cholesterol, cholesterol esters, free fatty acids and trace quantities of ethanolamine glycerophosphatides and serine glycerophosphatides, while the methanol eluate contained lecithin, lysolecithin and sphingomyelin plus a small amount of water soluble nonlipid material (mainly sodium chloride). Ethanolamine glycerophosphatides and serine glycerophosphatides were not detected in the methanol fraction. Lecithin and sphingomyelin were not detected in the chloroform-methanol 4:1 fraction.

The methanol eluate was evaporated to dryness and treated by mild alkaline hydrolysis. A sufficient quantity of 0.2 N KOH in methanol was added to the dried methanol fraction in a glass stoppered graduated cylinder to make a final concentration of 2 mg of lipid per ml and saponification was allowed to proceed at room temeprature for 17 hours after flushing the cylinder with nitrogen and tightly stoppering it. An equal volume of 2 N aqueous hydrochloric acid was then

added to the methanol phase. The methanol phase (now turbid) was extracted 3 times with 3 volumes of chloroform. The chloroform extract was separated and evaporated to dryness. It contained free fatty acids released from lecithin and lysolecithin plus unhydrolyzed sphingomyelin. The chloroform extract was evaporated to dryness, dissolved in chloroform and reapplied to the same silicic acid column after prewashing the column with chloroform (200 ml). Fatty acids were eluted with 200 ml of chloroform-methanol 4:1, and sphingomyelin with 150 ml of methanol. Each eluate was evaporated to dryness and weighed on an analytic balance. The quantity of sphingomyelin was determined from this weight while the quantity of lecithin was estimated by multiplying by 1.4 the weight of free fatty acids released during saponification. This factor was employed because the weight of fatty acids released after saponification of lecithin is 70% of the molecular weight of lecithin. Inclusion of lysolecithin in the lecithin fraction causes this estimate to be only slightly lower than theory since the content of lysolecithin in serum is small; one-eighth that of lecithin(1,2).

On the average 97.5% of the quantity of phospholipids estimated to be present from lipid phosphorus determinations on 4 serum samples was recovered from the columns. The purity of each fraction was determined by paper chromatographic analysis as described previously(10). Fig. 1 illustrates results of one such separation and shows the absence of contamination of serum sphingomyelin with lecithin or lysolecithin.

The fatty acids released from lecithin (plus lysolecithin) were methylated using the boron trifluoride-methanol reagent(11). The methyl esters were analyzed by GLC on columns of 10% diethyleneglycolsuccinate as previously described(10,12). Fatty acids were identified on the basis of their retention time compared with known standard compounds. Each peak was quantified by triangulation and comparing the area with that obtained with known standards (NHI Standard F and a laboratory standard made up of pure 16:0, 16:1, 18:0, 18:1, 20:0, 21:0, 22:0, 23:0 and 24:0 acids). Since the detector response was not identical

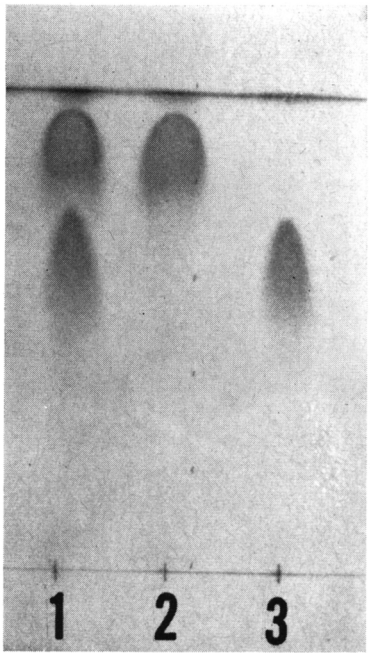

FIG. 1. Paper chromatography of serum lipids. Chromatogram of 1) a mixture of a lecithin standard and serum sphingomyelin; 2) the lecithin standard alone and 3) a serum sphingomyelin preparation, developed ascending on silicic-acid impregnated paper in chloroform-methanol 7:3 and stained with p-rosanaline(10).

for each acid, correction factors obtained from analyses of the above standard mixtures were employed to compensate for these variances. It was found that quantitative results with National Heart Institute Fatty Acid Standard F, and the laboratory standard agreed with the stated composition with a relative error of less than 5% for major components (> 10% of the total mixture) and less than 7% for minor components (< 10% of the total mixture).

Sphingomyelin fatty acids were analyzed as follows. Sphingomyelin was transferred to a test tube, dried under nitrogen, and a sufficient quantity of 2 N aqueous HCl was added to give a concentration of sphingomyelin of 2 mg/ml. The tube was sealed and heated for 2 hours at 120°. These conditions have previously been found to result in the complete release of fatty acid from sphingolipids(12,13). The completeness of hydrolysis was also checked during the analyses by weighing the purified fatty acids released after hydrolysis and by paper chromato-

TABLE II. Fatty Acid Composition of Serum Lecithin Plus Lysolecithin (as % of Total Fatty Acid Composition).

Acid	Subject 1	2	3	4	5	6	7	8	9	10
14:0	.3	.3	.3	.4	.5	.4	1.7	.4	.3	.3
15:0	.3	.2	.2	tr	.3	.1	.4	.1	.2	.2
16:0	33.3	38.1	36.4	38.1	35.8	37.8	39.6	35.4	38.2	36.2
16:1	.4	tr	.7	tr	.5	.3	tr	tr	tr	1.0
17:0	.3	.2	.2	.2	.2	.1	.2	.3	.3	.2
18:0	14.0	12.7	10.3	12.9	10.8	13.6	13.7	15.5	15.1	13.7
18:1	12.3	12.5	12.6	10.9	13.5	10.1	10.6	12.0	8.6	10.1
18:2	23.5	26.5	25.2	24.1	23.5	22.9	22.6	26.1	20.6	21.7
20:3ω3*	3.1	1.7	2.4	1.7	1.7	1.8	2.6	2.3	2.3	2.6
20:4	8.5	5.8	11.7	9.6	11.0	9.9	6.8	7.1	11.3	12.5
20:5	.8	.3	tr	tr	.3	.2	.4	tr	.9	tr
22:6	3.3	1.7	tr	2.1	1.9	2.6	1.4	1.1	2.2	1.9
Saturates	51.8	51.5	47.4	51.6	47.6	52.0	55.6	51.7	54.1	50.6
Unsaturates	48.2	49.5	52.6	48.8	52.4	48.0	44.4	48.3	45.9	49.4

* Tentative identification.

graphic examination of the hydrolysis products. After hydrolysis the liberated fatty acids (plus some sphingosine) were extracted from the aqueous phase with chloroform and the chloroform extract was evaporated to dryness. The fatty acids (plus some sphingosine) were refractionated using silicic columns as described above, eluting fatty acids with chloroform (200 ml) and sphingosine with methanol (150 ml). Sphingomyelin fatty acids were evaporated to dryness and methylated using boron trifluoride-methanol and analyzed on 10% diethyleneglycolsuccinate columns and 3% Apeizon L columns(12,13).

Results and discussion. The fatty acid composition of serum lecithin (plus lysolecithin) is given in Table II. The major fatty acids were 16:0, 18:1 and 18:2 while 20:4 and 22:6 were minor components. The ratio of saturated fatty acids to unsaturated fatty acids was very close to 1.0 in each lecithin sample. The long chain fatty acids present in sphingomyelin were not detected in lecithin.

The fatty acid composition of sphingomyelin is presented in Table III. The major fatty acids were 16:0, 18:0, 22:0, 24:1 and 24:0. A 25 carbon fatty acid was also present in amounts usually less than 0.1% of the total. The results, when averaged, corresponded with those reported by Sweeley for pooled human serum(5). No apparent differences were noted in the fatty acid composition of these lipids as a function of age. The serum sphingomyelin fatty acids were similar to those of sphingomyelin from brain(13,14) in that large proportions of long chain saturated and monounsaturated fatty acids were present. These two sphingomyelins differed from one another in that brain sphingomyelin contained predominantly stearic acid while serum sphingomyelin contained predominantly palmitic acid. The polyunsaturated fatty acids present in lecithin were absent in sphingomyelin. We agree with Sweeley(5) that reports of linoleic acid as a fatty acid of sphingomyelin(3,4) probably result from contamination of sphingomyelin with lecithin.

A comparison of Tables II and III indicates that the fatty acid compositions of sphingomyelin varied from subject to subject more than did those of lecithin. Direct statistical comparison was possible for 16:0 and 18:0, the major fatty acids in each lipid class. An analysis of the variance associated with differences between individuals revealed that both 16:0 and 18:0 were significantly more variable in sphingomyelin than in lecithin ($P>0.01$), with the variance for 16:0 being much larger than that for 18:0. Whether this variability will be borne out by a study of a larger number of individuals is being investigated.

Summary. A procedure is described for the isolation and characterization of the fatty acids of lecithin (plus lysolecithin) and sphingomyelin from blood serum. This procedure was employed for analysis of these fatty acids in 10 normal adult humans. The results indicate that serum lecithin is comprised of an approximately equal proportion of saturated

TABLE III. Fatty Acid Composition of Serum Sphingomyelin.

Acid	Subject 1	2	3	4	5	6	7	8	9	10	Avg	Pooled serum*
14:0	1.0	.8	1.1	.6	1.0	.9	.6	.8	.3	.9	.8	.7
15:0	tr	.2	.4	tr	.3	.1	.1	.2	.2	tr	.2	.2
16:0	41.0	35.6	39.6	58.7	48.7	64.0	28.4	60.1	56.2	55.8	48.8	41.7
17:0	tr	.2	.4	tr	.7	.2	.2	.2	.2	tr	.2	.6
18:1	.9	1.2	2.4	1.6	3.7	3.4	1.2	1.7	2.9	.8	2.0	.7
18:0	7.3	9.2	16.7	15.9	7.7	6.8	5.6	5.5	8.1	19.5	10.2	9.4
19:0	.9	.3	.9	.7	.1	.7	.8	1.5	1.4	tr	.7	.3
20:0	1.8	1.9	2.4	2.1	2.8	1.6	1.8	1.2	1.4	1.2	1.8	3.9
21:0	.6	.3	.4	tr	.3	tr	.5	.4	1.0	.9	.4	.3
22:1	.5	tr	tr	.2	tr	1.5	tr	tr	tr	.6	.3	.4
22:0	7.4	9.5	9.2	5.3	10.0	4.4	12.3	4.6	5.9	2.9	7.2	12.1
23:1	tr	tr	tr	.4	.3	tr	tr	tr	tr	2.6	.3	.5
23:0	5.6	6.3	4.9	2.6	4.1	1.4	9.9	5.2	3.5	7.3	5.1	5.1
24:1	23.0	22.1	12.2	7.7	10.8	11.2	21.6	12.2	8.6	5.2	13.5	14.1
24:0	10.0	12.5	9.0	3.9	8.5	3.0	17.0	6.4	10.3	2.3	8.3	8.9
25:0	tr	tr	tr	tr	1.1	.8	tr	tr	tr	tr	.2	.4

* Pooled human serum analyzed by Sweeley(5).

and unsaturated fatty acids while sphingomyelin contains a series of saturated and monounsaturated fatty acids ranging from 14 to 25 carbon atoms. The major fatty acids of lecithin were 16:0, 18:0, 18:1 and 18:2 while the major sphingomyelin acids were 16:0, 18:0, 22:0, 24:1 and 24:0. There were no apparent age-dependent changes in the fatty acid composition of these lipids (16-56 years). The fatty acids of sphingomyelin appeared to vary in composition from subject to subject more than those of lecithin.

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Effects of Trypsin and Chymotrypsin on Blood Glucose *in vivo* and Glucose Uptake *in vitro*.* (30322)

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Rieser and Rieser(1) have described an *in vitro* insulin-like activity of several proteases and have reported that trypsin and chymotrypsin are particularly active in promoting glycogen synthesis and the accumulation of 3-0-methyl glucose. In addition they have reported that insulin has proteolytic properties(2). It seemed reasonable that if the insulin-like *in vitro* effects of these pro-

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