toward invading leucocytes as the cells responding chemotactically to injection of antigen in the cornea. The possibility arises, therefore, that the cells of the cornea are merely the seat of the allergic response, and play either a minor role or none at all in actually eliciting it.

Summary. Injection of protein antigens in complete Freund's adjuvant into the footpads of guinea pigs produces a hypersensitivity which induces allergic reactions in the cornea both during the delayed and Arthus phases of the allergic response. This corneal reaction is similar to the intracutaneous delayed type in that its specificity is oriented toward the protein portion of a conjugate and not toward the hapten.

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Fatty Acids of Human Platelet Phosphatides.* (26663)

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The development of gas-liquid chromatography (GLC) provides a highly sensitive method for separation of fatty acid mixtures and identification of individual components (1,2). Studies of the fatty acid composition of a wide variety of lipids have been reported (3,4). Since platelet phosphatides exhibit specialized activity in the generation of blood thromboplastin(5) it is of interest to determine their constituent fatty acids by means of GLC.

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Materials and methods. Blood was collected(5) for platelet isolation from 25 subjects without apparent hematologic disorder. The phospholipids were extracted(5) and pooled. One hundred fifty-nine mg of crude phospholipid were chromatographed on a silicic acid column and the 150 individual fractions identified by paper chromatography(5). Each fraction was esterified by transmethylation in superdry methanol followed by sublimation according to Stoffel *et al.*(6). The yield of methyl esters from the sublimation cold finger was at least 95%

and colorless in petroleum ether solution. The GLC pattern of each silicic acid column fraction was determined with a Barber-Colman Model 10 chromatograph, equipped with a radium detector cell operated at 1000 volts, and a sensitivity of 1×10^{-7} amps. The methyl esters derived from the ascending and descending limbs of the 2 main silicic acid column peaks were pooled since they showed similar patterns. Argon provided the mobile phase at an inlet pressure of 35 psi with a flow rate at the column outlet of 200 ml per minute. To remove contaminants in the carrier gas, the tubing from the argon tank to the column was filled with 18 g of Molecular Sieve type 5-A(7). A U-shaped pyrex glass column, 6 feet in overall length with an internal diameter of 4 to 5 mm was employed. The stationary phases were adipate and succinate polyesters of ethylene glycol, 13.3% and 15% by weight respectively on 80-100 mesh Chromosorb W†. The "flash heater" was operated at a temperature of 250°C and detector cell at 230°C. The adipate column was held at 173°C, the succinate column at 180°C. Each column was conditioned at 197°C using a pressure of 40 psi for 4 days to minimize "bleeding." Percentage composition of the column eluate was determined by triangulation(1). Linearity of the detector response was evaluated by examining eluates of known purified fatty acid methyl esters.† In general, a difference of from 2 to 6% was noted between the amount applied and that eluted from the column. The chain length of fatty acids and their degree of unsaturation was determined by comparison with known standards and the retention times relative to methyl stearate, as reported by Farquhar *et al.*(1).

Since phosphatidylethanolamine (PE) and phosphatidylserine (PS) could not be separated adequately on the silicic acid column, the methyl esters of these phosphatides were examined together. PE and PS have previously been shown to represent the fraction of the platelet phospholipid active in clotting (8). Lecithin and sphingomyelin were also incompletely resolved from each other and studied as a mixture. They do not possess clot promoting activity(8).

Results. The pattern of the silicic acid column separation follows our previous reports(5,8). PE and PS predominate in the first 20 fractions, whereas lecithin and sphingomyelin form the bulk of tubes 21-150. The results of GLC on the methyl esters of the pooled tubes 7-10 and 11-16 are shown in Table I. These represent fractions on the ascending and descending limb of the PE-PS peak; tube numbers 27-35 and 36-150 in the Table are similar areas in the lecithin-sphingomyelin elution. Only the findings with adipate polyester at 173°C are given, but results with succinate polyester are similar. The adipate column had an efficiency of 2400 theoretical plates at methyl stearate (retention time 33 minutes). To simplify nomenclature, the shorthand designation(9) has been employed. Although approximately 37 components can be resolved in each chromatogram, many are minor and cannot be identified. These account for less than 2% of the total, and for purposes of clarity, are not shown in the Table. Values are reported as per cent of total methyl esters.

The principal fatty acids in the PE-PS fractions are arachidonic (45, 43%), stearic (22, 27%) and oleic (19, 13%). The lecithin-sphingomyelin elution shows essentially 2 patterns: In the early phase the primary fatty acids are oleic (45%), palmitic (23%), and stearic (18%) with a limited amount of linoleic (4%). These proportions change in the descending limb of the peak so that palmitic acid (64%) predominates; oleic (16%), stearic (6%) and linoleic (1%) decrease markedly. Distribution of the major fatty acids in the platelet phospholipids is shown in Fig. 1.

The dimethyl acetal derivatives of tetradecanal, hexadecanal, and octadecanal are noted in the PE and PS fractions. These represent the acetals formed on methylation of the aldehydogenic unit of plasmalogens (10). These phosphatides were previously noted to occur mainly in the PE and PS peaks and only trace amounts were detected

† Obtained from Applied Science Laboratories, State College, Pa.

TABLE I. Gas Chromatography of Platelet Phosphatide Fatty Acids Using EGA Polyester at 173°C as Stationary Phase.

Familiar name	Shorthand designation	Retention time relative to methyl stearate	% of total fatty acids			
			PE-PS fractions		Lecithin-sphingomyelin fractions	
			7–10	11–16	27–35	36–150
Lauric	12:0	.137	.2	.3	.3	.3
	Unknown	.199	.4	.5	.5	1.3
	14:0 DMA*	.222	1.1	1.8	.2	.6
Myristic	14:0	.274	.8	1.3	.2	.2
	16:0 DMA	.430	2.3	3.1	.05	.1
Palmitic	16:0	.496	1.1	1.1	23.8	64.1
Palmitoleic	16:1	.550	1.1	2.2	.4	.51
	Unknown	.610	.2	.3	.1	.24
	17:0	.695	.1	.1	.2	.26
	Unknown	.800	.2	.4	.03	—
	18:0 DMA	.900	.1	.1	.02	—
Stearic	18:0	1.00	22.4	27.7	18.6	6.5
Oleic	18:1	1.12	19.7	13.8	45.0	16.4
Linoleic	18:2	1.36	2.1	1.2	4.5	1.8
Arachidic	20:0	1.97	.2	.2	.8	1.3
	20:1	2.16	.2	.1	.6	.7
	20:3	3.00	.2	.3	.4	—
Arachidonic	20:4	3.32	45.6	43.5	3.1	1.8
	20:unsat.	3.87	.2	—	.8	3.2
	20:5	4.22	.3	.1	.2	—
	22:unsat.	6.50	.8	1.0	.2	—
	22:5	8.35	.3	.4	.3	.7
	22:6	9.30	.4	.5	—	—

* Dimethyl acetal of fatty aldehyde.

in the lecithin-sphingomyelin fractions(5, 11).

Discussion. The PE-PS fractions of platelet phosphatides can substitute *in vitro* for the entire platelet in formation of blood thromboplastin(5,8). Arachidonic acid represents approximately one-half of the fatty acid moiety in these eluates. Stearic and oleic acids are also major constituents of platelet PE and PS. The association of plasmalogens with the fractions promoting thromboplastic activity is again noted(5). In the lecithin-sphingomyelin peak, arachidonic acid virtually disappears. This striking reduction is of interest since clotting activity is absent in these fractions(5,8). Although it has been generally assumed that the ethanolamine or serine residue is responsible for the clotting activity of platelet "cephalin"(5), these results suggest that fatty acid moiety may be of considerable or even primary importance. Studies on the relationship of unsaturated fatty acid moieties to thromboplastic activity have been reported by Rouser and Schloredt(12) and Wallach *et al.*(13). When synthetic phosphatidylethanolamine containing 2 saturated fatty acids (L-alpha-(dimyristoyl) cephalin)‡ was

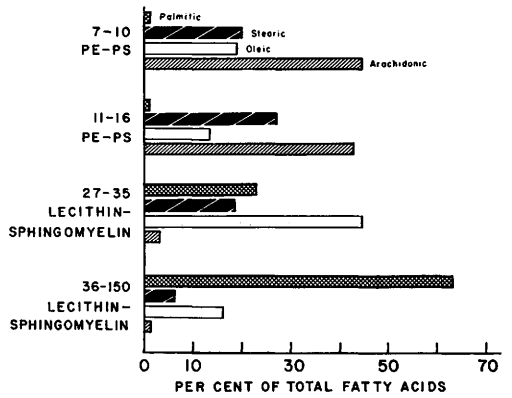

FIG. 1. Distribution of major fatty acids in blood platelet phosphatides as separated by silicic acid and gas-liquid chromatography. Fractions 7-10 and 11-16 are active in formation of blood thromboplastin, whereas 27-35 and 36-150 are not.

‡ Kindly provided by Dr. Erich Baer.

tested in the thromboplastin generation test, it was completely inert in all concentrations studied. It must also be pointed out that the exact relationship of plasmalogens to blood coagulation has not been clarified.

The results of GLC analyses of fatty acid composition of red cell phosphatides recently made by Farquhar *et al.* show an overall similarity to the patterns of platelets.§ This is of importance because it has been found that red cell phospholipids can promote formation of blood thromboplastin(14,15).

Summary. The fatty acids of blood platelet phosphatides have ben studied by silicic acid, and gas-liquid chromatography. PE-PS fractions contain primarily arachidonic, stearic and oleic acids. Lecithin-sphingomyelin eluates contain principally palmitic, stearic and oleic acids. These components may be quantitatively related to the specialized function of platelets in blood coagulation.

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Serologic Studies of Rabbit Antibodies to Rabbit Submaxillary Glands.* (26664)

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Experimental evidence has accumulated during the last few years in reference to the pathogenesis of chronic non-specific thyroiditis(1). It has been shown that experimental animals such as rabbits, dogs and guinea pigs when immunized with extracts of thyroid glands of the respective species frequently develop thyroid specific antibodies demonstrable either by the usual serological tests as circulating antibodies or by technics employed to demonstrate delayed hypersensitivity, or both(2). They also frequently show histological evidence of thyroiditis.

Recently attention has been drawn to the pathogenesis of Sjögren's syndrome, a clinical triad characterized by keratoconjunctivitis sicca, xerostomia and also rheumatoid

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