peronii copulate only in autumn and that sperm are stored in the oviducts during winter.

This pattern of reproduction, involving obligatory sperm storage, has not yet been described for any other reptile. We suggest that its adaptive value is that, by courting and copulating in the autumn, the females can ovulate earlier in the spring and so produce their young earlier. This gives the young a better chance to feed and grow before the winter.

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29 April 1968

Ionic Interaction of Sulfatide with Choline Lipids

Abstract. *Aqueous systems of sphingomyelin-sulfatide and lecithin-sulfatide were compared with aqueous systems of the individual lipids. The acid capacity of the mixed lipids increased, a result of the formation of an ionic bond between the sulfate of one molecule and the positive nitrogen of the other, making the phosphate available for direct titration. Cholesterol reduces this ionic interaction, probably because of the increased spacing of the ionized groups.*

We now report that in aqueous dispersions ionic binding occurs between polar groups of sulfatide and choline phospholipids. This finding may be of importance in the understanding of the cohesive forces operating in biological membranes. The structural integrity of biologic membranes has been attributed to the forces acting between the long hydrocarbon chains of neighboring lipid molecules when the chains are parallel to each other (*1*). There is evidence that hydrophobic bonding between the hydrocarbon chains of lipids with hydrophobic portions of a structural membrane protein may occur (*2*). In addition, there may be bridging of negative-charge sites in the aqueous phase by multivalent cations or by positive sites of proteins. Pethica (*3*) calculated that the forces potentially present between the polar groups of adjacent lecithin molecules in a monolayer are a major factor in the stability of sheetlike structures. But the existence of intermolecular bonding in lecithin has been questioned by others (*4*). Our experiment offers direct evidence of ionic binding between lipids.

When phospho- and glycolipids are dispersed in water, they form lamellar structures 44 to 60 Å in width; these structures are probably lipid bilayers (*5*). In aqueous dispersions of the acidic lipids—phosphatidylserine, phosphatidic acid, phosphatidylinositol, and sulfatide (*6*)—all of the acidic groups are available for titration or ion binding; this suggests that the lipids are oriented with the ionic group exposed to the aqueous phase. Thus, these lipid aggregates or micelles may have characteristics analogous to those of the lipid portion of the biological unit membrane.

The lipids used in our experiments include sphingomyelin purified from beef brain as described (*7*); sulfatide (*8*) converted to the sodium salt, as reported (*6*); egg lecithin (*9*) purified by silicic acid chromatography; and cholesterol. All of the lipid preparations were pure, as judged from thin-layer chromatograms as described below. Analyses of the sphingomyelin and sulfatide preparations used have been reported (*6, 7*).

Aqueous dispersions of the lipids were produced (*6*) by placing the lipid or mixture of lipids in a mixture of chloroform and methanol (2: 1 by volume) in a 20-ml glass tube. The organic solvent was evaporated, 5 ml of water (conductivity $< 10^{-6}$ ohm^{-1} cm^{-1}) was added, and the system was exposed to gentle ultrasonic radiation.

The aqueous systems were titrated at 24° ± 1°C at least twice with an expanded-scale *p*H meter (Corning model 12), and combined glass and reference electrodes were used; stirring at a very slow constant rate was provided, and a rapid stream of nitrogen was passed over the system. Additions of 1 to 3 μl of 0.100*N* HCl or NaOH were made, and sufficient time was given for the system to reach a stable *p*H. All titrations were corrected.

After completion of a titration, a 0.1-ml sample was evaporated to dryness and dissolved in a mixture of chloroform and methanol (2 : 1 by volume). A thin-layer chromatogram was obtained with silica gel G (Merck), developed with a system consisting of chloroform, methanol, water, and ammonia (29 percent) (70 : 30 : 4 1 by volume); it was then stained with iodine vapor and charred with sulfuric acid. Lecithin systems showed a small amount of degradation (<5 percent); therefore, titrations were not carried into the alkaline regions.

The increase in titration capacity of the sphingomyelin and sulfatide mixtures as compared to individual lipids is shown in Fig. 1. The small titration capacity of sulfatide from *p*H 7 to 3.5 is due to the strong acid ionization of the sulfate, whereas in sphingomyelin or lecithin the ionic bonding of the phosphate and positive nitrogen limits its acid capacity. The mixed sulfatide-sphingomyelin systems exhibit titrations comparable with that of phosphatidyl inositol (*6*) (Fig. 1). The increased acid capacity of lipids in the mixed systems over their individual capacities can result from the formation of an ionic bond between the ionized sulfatide molecule with the positive nitrogen in the choline portion of sphingomyelin.

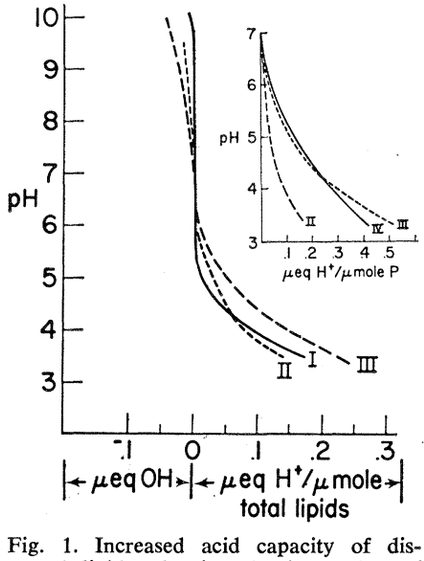

Fig. 1. Increased acid capacity of dispersed lipids, showing the interaction of sulfatide with sphingomyelin. Each system containing the lipid dispersed in 5 ml water was titrated with 0.100*N* HCl or NaOH. Curve *I*, 7.63 μmole of sodium sulfatide; curve *II*, 10.0 μmole of sphingomyelin; curve *III*, 7.9 μmole of sphingomyelin + 7.9 μmole of sodium sulfatide, mixed and dispersed. The inset compares *III*, a 1 : 1 complex of sphingomyelin-sulfatide with *IV*, phophatidylinositol, and *II*, sphingomyelin alone. The similarity of *III* and *IV* indicates that the ion titrated in the complex is the phosphate of sphingomyelin, made available by the interaction of the sulfate with the positive choline.

Table 1. Acid capacity of 1:1 sphingomyelin-sulfatide or 1:1 egg lecithin–sulfatide complexes in several mixtures after correction for the contribution of the excess component.

Composition of systems (μmole)				Acid capacity (μeq H^+/μmole complex)	
Sodium sulfatide	Sphingomyelin	Egg lecithin	Cholesterol	*p*H 7.0–4.0	*p*H 7.0–3.5
7.9	3.2			0.25	0.32
7.6	4.4			.28	.50
7.9	9.5			.30	.45
7.4	8.9			.34	.46
4.4	7.8			.28	.39
8.5	8.5		4.2	.21	.37
8.5	8.8		8.5	.17	.24
8.9		9.3		.20	.31
Comparison with single lipids				*(μeq H^+/μmole lipid)*	
Sphingomyelin				0.083	0.14
Egg lecithin				.044	.058
Sodium sulfatide				.085	.16
Phosphatidylinositol				.28	.37

This frees the phosphate of the choline lipid, which then may be titrated in a manner similar to that for a lipid with an ionizable phosphate, like phosphatidylinositol. This reaction takes place only when the lipids are present in the same particle, as shown when a dispersion of sphingomyelin was mixed with one of sodium sulfatide. Titration showed no interaction of the lipids after 24 hours at 25° or 40°C.

Sphingomyelin-sulfatide systems were prepared with varying mole ratios of these lipids. The acid capacity between *p*H 7 and 3.5 was determined. Again, assuming a complex is formed with a 1 : 1 mole ratio, the capacity of the excess component was deducted from the total which was then divided by the number of moles of complex. These results may be compared with the individual components and with the sodium salt of phosphatidylinositol. The acid capacity of lecithin-sulfatide mixtures was slightly less than that of the sphingomyelin-sulfatide, possibly because the larger area occupied by egg lecithin (*10*) increases the distances between ionic groups.

The incorporation of cholesterol or cerebroside into sphingomyelin or lecithin systems did not change the titration characteristics of these lipids. Cholesterol, however, reduces the titration capacity of sphingomyelin-sulfatide mixtures. As the mole ratio of cholesterol is increased, the acid capacity of the complex decreases (Table 1), an indication that cholesterol molecules may be interposed between the sphingomyelin and sulfatide, increasing the distance between the charged groups.

The choline phospholipids, lecithin and sphingomyelin, are important constituents in many biological membranes. Within such systems, ionic binding between the choline phospholipids and anionic lipids probably occurs, thus adding to the stability of these membranes.

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18 March 1968; revised 17 May 1968

Pyrrhotites: Synthetics Having Two New Superstructures

Abstract. *Synthetic pyrrhotites $Fe_{1-x}S$, synthesized at various compositions and temperatures, show the presence of two new superstructures based on the hexagonal subcell of the NiAs type (axes* A *and* C*): one, in the range* $1-x=0.89$ *to* 0.93*, has* $a = 90$A *and* $c = 3$C*; the other, in the range* $1 - x = 0.935$ *to* 0.975*, has* $a = 2$A *but* c *irrationally related to* C*, varying with composition.*

Because of its common occurrence and unusual magnetic properties, pyrrhotite $Fe_{1-x}S$ has been extensively studied, but many important questions regarding its phase relations and crystallography remain unanswered. Three different types of pyrrhotite have been confirmed as low-temperature stable phases (*1*, *2*), all of which are superstructures of the NiAs type of structure, whose cell dimensions are *A*, about 3.45 Å; *C*, about 5.8 Å. They are

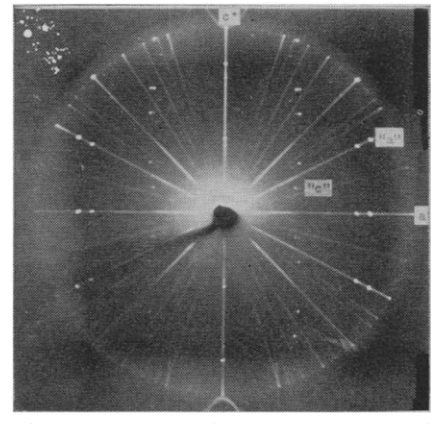

Fig. 1. Precession photograph $(010)_0$* of 3*C*-type pyrrhotite ($Fe_{0.90}S$) quenched from 300°C; Fe$K\alpha$, $\mu=25$ deg, 190 hours. The *a** and *c** axes have been indicated as well as the "*a*"-type and "*c*"-type reflections.

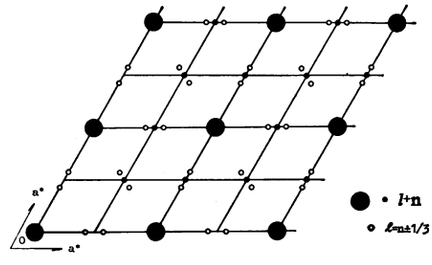

Fig. 2. Reciprocal lattice projected on (001)* of the 3*C* type of pyrrhotite (not to scale). Large solid circles, "*a*"-type reflections; small solid circles, "*b*"-type reflections; open circles, "*c*"-type reflections (see text).