Table 1. Daily determinations of luteinizing hormone activity in plasma throughout the menstrual cycle in ten women. One mμg of the reference preparation equals 8 milliunits of the Second International Reference Preparation for human menopausal gonadotrophins. Values for differences of means are significant at $P < .05$ in the luteal phase for all subjects except the one with a 34-day cycle.

Peak of plasma LH (day)	Peak	LH in plasma (mμg/ml)					
		Follicular phase			Luteal phase		
		No.	Range	Mean ± S.E.	No.	Range	Mean ± S.E.
				26-day cycle			
18	12.6	13	1.6–6.2	2.7 ± .3	7	1.0–1.8	1.3 ± .2
15	8.0	12	1.7–2.4	2.1 ± .09	8	1.2–2.4	1.6 ± .2
				29-day cycle			
16	5.2	14	1.6–2.4	1.8 ± .07	11	1.1–1.9	1.5 ± .08
14	7.3	12	1.5–2.2	1.8 ± .07	13	1.3–2.2	1.6 ± .09
13	8.8	10	1.5–2.6	2.0 ± .11	14	0.9–2.2	1.4 ± .1
				30-day cycle			
17	11.2	15	1.1–2.4	1.9 ± .09	11	0.9–2.8	1.4 ± .05
21	7.0	13	1.4–2.4	1.8 ± .04	6	1.0–1.6	1.3 ± .12
				31-day cycle			
16	6.8	14	0.9–2.3	1.5 ± .1	14	0.9–2.2	1.2 ± .09
				33-day cycle			
16	6.4	10	1.4–2.1	1.7 ± .08	15	0.7–1.8	1.2 ± .07
				34-day cycle			
20	19.2	16	1.2–3.0	2.2 ± .1	14	1.4–3.8	2.1 ± .09

years of age. Heparin (0.1 ml of a solution containing 1000 unit/ml) was used as anticoagulant, and plasma was separated from cells in a refrigerated centrifuge and frozen until assayed. Basal body temperatures were recorded.

Radioimmunoassay was done, with 300-μl samples of plasma (*3*). Evidence for the specificity of the method and for the correspondence of values determined simultaneously by bioassay and radioimmunoassay has been presented (*4*). Results of determinations are given in millimicrograms of the human luteinizing hormone (HLH) preparation used as standard in the assay. One mμg of this preparation is equivalent to 8 milliunits of the Second International Reference Preparation for human menopausal gonadotrophins.

Around midcycle, a sharp LH peak with values from 5.2 to 19.2 mμg per milliliter of plasma and lasting for less than 24 to 48 hours occurred in every subject during a period from 3 to 7 days in which values were more than three standard deviations in excess of mean values before and after the peak. In one instance, a second, lower peak was seen on the 3rd day of menses, prior to the higher peak.

There was no consistent relation between length of cycle and either the day of the peak of plasma LH or the interval between peak and next menses (Table 1). The peak occurred on the day of the thermal nadir in basal body temperatures in those subjects where this day could be identified.

Because samples drawn at 24-hour intervals did not define accurately the duration of the peak level, samples are being taken at intervals of 6 to 8 hours in current studies. In three subjects, the peak values persisted less than 16 hours, and the samples taken at 8:00 a.m. showed no significant variation from maximum values on the day of the peak. Hence, variability in peak levels was probably real and not an artifact of the frequency of sampling.

Without exception, means of daily values before the peak at midcycle were greater than those after it, and these differences were statistically significant in nine subjects. Statistically valid differences in LH levels in follicular and luteal phases of the cycle have not been reported previously in studies on urine (*1*, *2*) nor in a study done by bioassay on single samples of plasma taken during follicular and luteal phases in three women (*5*). In concentrates of urine, the limited quantity of LH recoverable, frequently inadequate to demonstrate any biological or immunological activity in a single 24-hour collection (*1*, *2*), and the consequent necessity for pooling might obscure small differences which are appreciable by the radioimmunoassay method; this method provides increased precision and feasibility of determinations on multiple samples collected on a single day.

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Ethanolamine Phosphoglycerides: Effect on the Properties of Myelinoid Lecithin Water Systems

Abstract. *The amount of swelling solution trapped when mixtures of ethanolamine and choline phosphoglycerides were dispersed in 0.145*M *glucose-C^{14} is dependent on, but not linearly related to, the amount of ethanolamine phosphoglyceride in the mixture. The leakage of swelling solution out of such myelinoid lipid-water dispersions was, however, linearly related to the proportion of ethanolamine phosphoglyceride.*

The presence of lipid as an integral part of biological membrane architecture has seriously complicated explanation of the selective permeability to ions and small polar molecules of such membranes. Postulated mechanisms have generally involved the presence of pores or carrier molecules or both. Recently, however, Bangham and others (*1*) have shown that myelinoid lipid-water systems of pure choline phosphoglycerides (CPG) exhibit a selective permeability and a response to biologically active steroids and anaesthetics that is qualitatively very similar to natural membranes.

The structures of such liquid crystals are generally accepted as being spheres or toroids consisting of a series of bimolecular leaflets of lipid separated by aqueous compartments. The thickness of these aqueous compartments

and the permeability of the system appear to be controlled by the sign and magnitude of the charge at the lipid-water interface. In natural membranes, CPG, which is electrically neutral at physiological *p*H, is the major component comprising roughly 50 percent of the total phospholipid, but the membrane usually contains considerable proportions of ethanolamine phosphoglyceride (EPG) which has a net negative charge at *p*H around 7 (*2*).

It was therefore of interest to study mixtures of EPG and CPG and thereby offer evidence to support or reject the oft-repeated speculation that the lipid composition of the membrane will exert considerable control over permeability of the natural membrane. Based on Bangham's work, one would predict an increase both in permeability and the size of the interlamellar space with increasing proportions of EPG.

Preparations of EPG that gave only a single spot in thin-layer chromatography were prepared by silicic acid chromatography (*3*) from lipids of *Phormia regina* (the black blowfly). The CPG was either similarly prepared, and contained a minor quantity of EPG, or it was pure commercial ovolecithin (General Biochemicals, Chagrin Falls, Ohio). Both preparations of CPG behaved in the same way. The lipids were swollen in 0.145*M* glucose-C^{14} (3551 count $min^{-1}/\mu mole^{-1}$), and the excess swelling solution was removed by dialysis against nonradioactive 0.145*M* glucose. All dialyses for a given experiment were carried out simultaneously in the same vessel to ensure uniformity of treatment. Chen's (*4*) analysis for phosphorus was used.

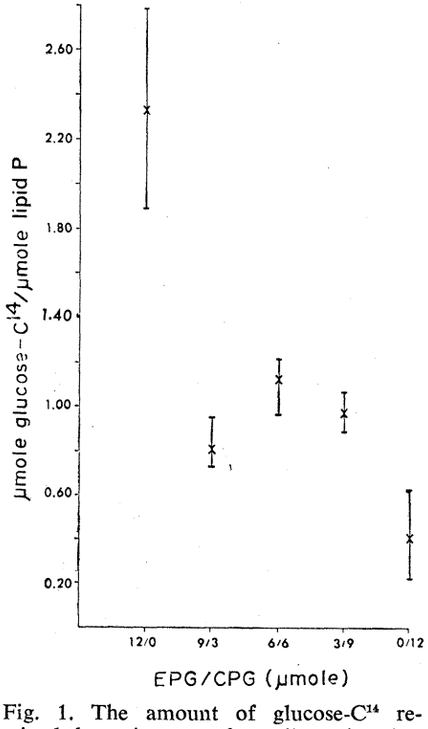

Fig. 1. The amount of glucose-C^{14} retained by mixtures of swollen phospholipids after dialysis for 24 hours against unlabeled isotonic glucose. Bars indicate the range of values.

Liquid crystals of EPG retained 2.33 μmole of glucose-C^{14} per micromole of lipid P after dialysis for 24 hours against repeated changes of dialyzing solution, whereas those formed from CPG retained only 0.41 μmole per micromole of lipid P. Dialysis controls which contained swelling solution but no lipid retained only 0.04 μmole of glucose-C^{14}.

The amount of swelling solution retained by mixtures of these two lipids, however, was only roughly proportional to the amount of EPG in the mixture (Fig. 1). Thus the amount of EPG increases the size of the aqueous compartment as predictable from theory, but the lipids appear to interact somehow, modifying the effect of opposed negative charges.

The size of the aqueous compartment as determined by the amount of swelling solution trapped was also determined with two natural phospholipid mixtures having radically different ratios of EPG to CPG. Complex lipids (methanol eluate from a silicic acid column after elution of the simple lipids with chloroform) of *Phormia regina* with an EPG : CPG ratio of 4.2 (*5*) trapped 0.88 μmole of glucose-C^{14} per micromole of lipid P, whereas complex lipid from *Gryllus bimaculatus* with an EPG : CPG ratio of 0.67 (*6*) trapped 0.4 μmole of swelling solution per μmole of lipid P. The lipid from swollen dispersions had the same qualitative composition as the original by thin-layer chromatography. Again the size of the aqueous compartment is only roughly proportional to the amount of EPG in the mixture; the additional modifying effect of the minor components of the natural mixtures must also be considered.

According to Bangham, the net negative charge induced by EPG should result in an increase in the permeability of the lipid bilayer. In order to obtain a measurable amount of leakage within a reasonable time, dicetyl phosphate (DCP) was added at a constant molar ratio to each lipid sample before swelling. After swelling, the concentration of untrapped glucose-C^{14} was reduced by five successive 30-minute dialyses in 500 ml of tracer-free 0.145*M* glucose solution. Leakage was then determined by suspending the dialysis sack in 10 ml of fresh unlabeled glucose solution and counting the glucose-C^{14} appearing in this 10 ml at the end of 30 minutes. An almost linear increase in leakage occurred with increasing proportions of EPG (Fig. 2.) Either the forces modifying the effect of negative charge on the size of the aqueous compartment do not affect permeability, or the additional negative charge supplied by DCP might override them. Whatever the reason for the smoothness of the curve, EPG increases the permeability of the lipid system.

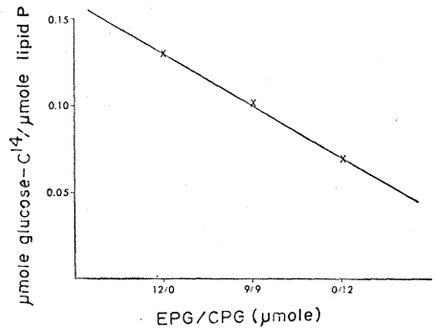

Fig. 2. Leakage of glucose-C^{14} out of swollen phospholipids containing dicetyl phosphate.

It has often been suggested that the class composition of the phospholipids in a biological membrane will affect its permeability characteristics, but few grounds existed for predicting the direction of the effect. The evidence presented here and Bangham's work both indicate that higher proportions of EPG in a mixture will increase the permeability. The data in Fig. 1 suggest that this control of permeability may be more complex than a simple consideration of charge would indicate.

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