to extramitochondrial) of 0.44 (Fig. 2), indicating a passive transport.

To detect the saturation of active transport of DMO, if present, we incubated respiring and phosphorylating mitochondria with large concentrations of unlabeled DMO. So that quantitation and distribution could be based on radioactive analysis, ^{14}C-DMO was added. The ratio of intra- to extramitochondrial DMO for six different extramitochondrial concentrations (0.9 to 29 $\times$ $10^{-2}M$) remained at 1.4, equivalent to a *p*H gradient (intra- to extramitochondrial) of 0.20 (Fig. 3), indicating absence of active transport.

During respiratory jump activated by Ca++ (0.6237 $\times$ $10^{-2}M$) (Fig. 2), the ratio of intra- to extramitochondrial DMO ratio was found to be 30, equivalent to a *p*H gradient (intra- to extramitochondrial) of 1.56. This is an expected change, because a release of H+ ions from the mitochondria and an alkalinity of the mitochondrial pellet have been shown under similar experimental conditions (*11*). Also, the *p*H gradient of 1.56 *p*H units in our studies is not far from that reported for rat liver mitochondria by Chance and Mela (*12*) who, using bromthymol blue, measured a *p*H gradient of about 1.00 *p*H unit under conditions somewhat similar to ours. Furthermore, the significant alkalinization during the Ca++ activation was neutralized with concomitant significant decreases in the ratio of intra- to extramitochondrial DMO (from 30 to 3.4) and in the *p*H gradient (from 1.56 to 0.3) when either 8.3 mM acetate or 1.7 mM phosphate were added, respectively, to mitochondria activated by Ca++ (*4*). These findings were also in agreement with those reported by Chance and Mela (*13*). In addition, these observations demonstrate that DMO anions are not carried along with the cations (Ca++) into the mitochondria and that distribution of intra- and extramitochondrial DMO is a function of intramitochondrial *p*H.

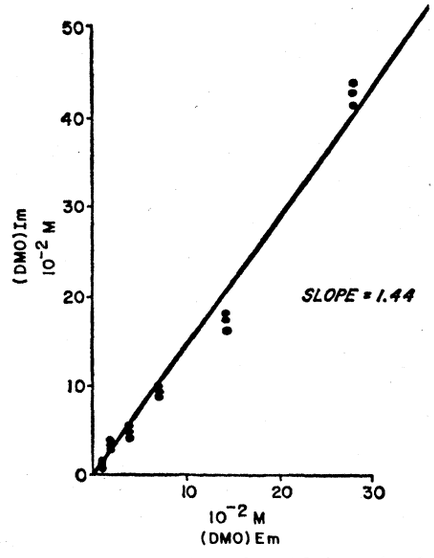

Fig. 3. Incorporation of DMO into simultaneously respiring and phosphorylating mitochondria. Beef heart mitochondria were isolated at 4°C in 0.33M sucrose containing tris chloride (0.05M, *p*H 7.5 at 4°C), and 28 mg of protein was incubated at 30°C for 1 minute. When examined in an Oxygraph (GME, Middleton, Wisconsin), these mitochondria had the following characteristics; respiratory control 2.2, P/O ratio (μmoles of adenosine triphosphate produced per μatom oxygen consumed) 3.2, and specific activity 0.082 μatom O_2 per minute per milligram of protein. The reaction medium contained: 1 μc of ^{14}C-DMO; 1 μc of ^{14}C-labeled carboxy dextran; 0.9 $\times$ $10^{-2}M$ to 29 $\times$ $10^{-2}M$ cold DMO; tris phosphate 0.002M; sodium pyruvate 0.004M; tris malate 0.004M; adenosine diphosphate 0.004M; $MgCl_2$ 0.020M; sucrose 0.208M; and tris chloride 0.05M, *p*H 7.5 in a total volume of 10 ml. In view of the fact that phosphate acceptor (ADP) is added to the reaction medium which contains in addition Mg^{++}, phosphate ions, and substrates, oxidative phosphorylation will predominate with about 20 percent maximum accumulation of phosphate and Mg^{++} (*10*). At the end of incubation the reaction mixtures were treated as in Fig. 1.

Our data indicate that DMO is passively transported into resting, respiring, and simultaneously respiring and phosphorylating mitochondria. Also, the similar ratio of intra- to extramitochondrial DMO for each functional state (with different concentrations of DMO in the medium) suggest no binding of DMO to mitochondrial constituents. Therefore, the DMO method should be valid for the measurement of intramitochondrial *p*H. In view of the fact that the mitochondrial membranes possess many of the structural and functional characteristics typical of cellular membranes (*14*), it is reasonable to assume that a similar behavior of cellular membranes exists with respect to DMO.

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References and Notes

1. J. M. Dietschy and N. W. Carter, *Sctence* **150**, 1294 (1965); T. C. Butler, *ibid.* **151**, 1564 (1966); J. M. Dietschy and N. W. Carter, *ibid.* **152**, 799 (1966).
2. Y. Hatefi, P. Jurtshuk, A. G. Haavik, *Arch. Biochem. Biophys.* **94**, 148 (1961).
3. ^{14}C-labeled carboxy dextran (molecular weight 60,000 to 90,000) and DMO-2-^{14}C (referred to as ^{14}C-DMO) were obtained from New England Corporation, Boston, Massachusetts.
4. For the validity of centrifugation, see S. Addanki, F. D. Cahill, J. F. Sotos, in preparation.
5. R. L. O'Brien and G. P. Brierly, *J. Biol. Chem.* **240**, 4527 (1965).
6. A. G. Bray, *Anal. Biochem.* **1**, 279 (1960).
7. D. Y. Poole, T. C. Butler, W. J. Waddell, *J. Nat. Cancer Inst.* **32**, 939 (1964).
8. E. Schram, in *Organic Scintillation Detectors*, E. Schram and R. Lombaert, Eds. (Elsevier Publishing Company, Amsterdam, 1963), p. 123; C. T. Peng, *Anal. Chem.* **32**, 1292 (1960).
9. A. G. Gornal, C. J. Bardawill, M. M. David, *J. Biol. Chem.* **177**, 751 (1949).
10. G. P. Brierly, E. Bachmann, D. E. Green, *Proc. Nat. Acad. Sci. U.S.* **48**, 1928 (1962).
11. C. S. Rossi, J. Bielawski, A. L. Lehninger, *J. Biol. Chem.* **241**, 1919 (1966).
12. B. Chance and L. Mela, *Proc. Nat. Acad. Sci. U.S.* **55**, 1243 (1966).
13. B. Chance and L. Mela, *Nature* **212**, 369 (1966).
14. W. Stoeckenius, *Ann. N.Y. Acad. Sci.* **137**, 641 (1966).
15. We thank R. Wright, P. Bartholomew, H. Headley, and P. D. Rearick for technical assistance; Drs. G. P. Brierly, A. J. Merola, C. D. Stoner, and W. J. Frajola, Department of Physiological Chemistry, The Ohio State University, for suggestions; Dr. B. Chance, University of Pennsylvania, for discussions; and Dr. L. G. Ginger, Baxter Laboratories, Morton Grove, Illinois, for supplying unlabeled DMO. Supported by USPHS grant NB-05114, FR-78, and American Cancer Society Institution grant IN-16H.

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Luteinizing Hormone Activity in Plasma during the Menstrual Cycle

Abstract. *Daily determinations of luteinizing hormone activity in plasma throughout a menstrual cycle in ten young women showed a sharp peak of activity lasting less than 48 hours around midcycle and higher mean values during the follicular phase than during the luteal phase in nine instances.*

Elevation in urinary excretion of substances with biologic (*1*) and immunologic (*2*) characteristics of luteinizing hormone (LH) occurs about the time of the rise in basal body temperatures in women with ovulatory menstrual cycles. When we used a sensitive, precise radioimmunoassay method for luteinizing hormone in plasma (*3*), we found a corresponding elevation in samples taken daily throughout a single menstrual cycle in ten young women. Mean values during the follicular phase significantly exceeded those during the luteal phase in nine instances.

Daily samples (5 ml) of venous blood were drawn before noon on the 1st to 3rd day of menses and every day thereafter until the 1st day of the next menses. The subjects were ten healthy young women from 19 to 27

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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References and Notes

1. J. W. McArthur, J. Worcester, F. M. Ingersoll, *J. Clin. Endocrinol. Metab.* **18**, 1180 (1958); M. Fukushima, V. C. Stevens, C. L. Gantt, N. Vorys, *ibid.* **24**, 205 (1964); K. L. Becker and A. Albert, *ibid.* **25**, 962 (1965); E. Rosemberg and P. J. Keller, *ibid.*, p. 1262.
2. L. Wide and C. Gemzell, *Acta Endocrinol.* **39**, 547 (1962); W. R. Butt and A. C. Crooke, *Proc. Roy. Soc. Med.* **57**, 851 (1964); T. Sato, R. B. Greenblatt, V. B. Mahesh, *Fertil. Steril.* **16**, 223 (1965).
3. W. D. Odell, G. T. Ross, P. L. Rayford, *Metabolism* **15**, 287 (1966).
4. ———, *J. Clin. Invest.*, in press.
5. J. Louchert, J. Truffert, J. Decourt, *Acta Endocrinol.* **49**, 293 (1965).

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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