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INDUCTION OF GEL-PHASE LIPID IN PLASMA MEMBRANE OF CHICK INTESTINAL CELLS AFTER COCCIDIAL INFECTION

J.E. THOMPSON [a], M.A. FERNANDO [b] and J. PASTERNAK [a]

[a] *Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1 and*
[b] *Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N2G 2W2 (Canada)*

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Summary

When chickens are infected with the coccidial parasite *Eimeria necatrix*, the plasma membrane of intestinal cells harbouring second-generation schizonts becomes refractory to mechanical shearing, hypotonic shock and ultrasonication. Plasma membrane from these infected cells was isolated to high purity as judged by enriched levels of ouabain-sensitive ($Na^+ + K^+$)-stimulated Mg^{2+}-dependent ATPase activity and sialic acid content, the lack of detectable cytochrome oxidase and glucose-6-phosphatase activities and electron microscopic analysis of the final preparation. Wide-angle X-ray diffraction patterns recorded from the isolated membranes revealed that during the later stages of parasite maturation the host cell plasma membrane acquires increasing proportions of gel-phase lipid. By contrast, purified membrane from isolated parasites is in a liquid-crystalline state. The transition temperature of host cell plasmalemma at 100 h postinfection is 61°C, about 20°C above physiological temperature. By contrast, liposomes of plasma membranes from infected cells undergo a thermal transition at about 28°C. The accumulation of gel-phase lipid in the host cell plasma membrane is not attributable either to an increase in the constituent ratio of saturated to unsaturated fatty acids or to a significant change in the cholesterol to phospholipid ratio. During the late stages of infection, the cells become stainable with trypan blue which suggests that the acquisition of crystalline phase lipid disrupts the permeability of the host cell plasmalemma.

Introduction

Under biologically active conditions the lipid bilayer matrix of cellular membranes is in a liquid-crystalline (fluid) state. A thermotropic transition

from this liquid-crystalline state to a gel phase can be induced in model membrane systems, purified membrane fractions and membranes of living cells by decreasing the temperature [1—4]. In addition, for some systems the phase properties and fluidity of membrane lipids can be manipulated experimentally by varying the concentration of precursor compounds in the growth media [5—11]. At an extreme, in the presence of specific fatty acid supplements some organisms can grow when 90% of the membrane lipid is in the gel phase [10, 12]. Other organisms maintain the liquid-crystalline state over a range of growth temperatures by regulating fatty acid incorporation into membrane lipid [13,14].

To date there are only a few documented instances of crystalline lipid formation in membranes under physiological conditions which have not entailed either manipulation of the growth medium or use of genetic variants. For example, during germination of bean, microsomal membranes of aging cotyledons acquire increased proportions of gel-phase lipid [15,16]. The transition to the gel phase is extensive and strikingly evident 48 h prior to abscission. Similarly, a conversion from the characteristic liquid-crystalline to gel phase has been observed in microsomal and chloroplast membranes of senescing leaf tissue [17] as well as in smooth microsomal membranes from aging batch cultures of the green alga *Scenedesmus quadricauda* [18]. In each of these cases the formation of gel-phase lipid is associated with the onset of cell deterioration and could conceivably contribute to the loss of intracellular compartmentalization that characterizes the period of autolysis preceding cell death.

In the present study physical and chemical properties of the plasma membrane of chick intestinal cells after infection with an obligate intracellular parasite were examined. Coccidial parasites induce dramatic changes in their host cells that are ultimately lethal. In chick intestinal cells infected with second generation schizonts of *Eimeria necatrix*, (i) the host cells undergo extensive hypertrophy with an eventual five-fold increase in surface area after penetration of the parasite; (ii) there is a 6—10-fold increase in the DNA content of nuclei of infected cells; (iii) the plasma membrane of the host cell becomes refractory to fracture by mechanical shearing, hypotonic shock and ultrasonication and (iv) extensive autolysis of the host cell cytoplasm occurs during the latter stages of infection [19,20]. The presence of such an unusual plasma membrane facilitated the development of a procedure for isolating infected cells at various times after the invasion of the parasite [21]. In this context, the following question arises: in what way has the status of the host cell plasma membrane been altered as a consequence of the intracellular infection? In the present study it is demonstrated, using X-ray diffraction analyses, that the plasma membrane of the host cell acquires increasing proportions of gel-phase lipid during the later stages of infection. Concomitantly, the membrane lipid of the developing parasites remains exclusively liquid crystalline at physiological temperature.

Materials and Methods

Membrane isolation. Both the breed of chickens and strain of *E. necatrix* used in this study have been described previously [22]. Prior to the oral admin-

istration of $1-5 \cdot 10^5$ sporulated oocysts, coccidia-free chicks were raised in isolation quarters for 3—10 weeks. Large numbers of first generation merozoites infect intestinal cells 66 h after the initial infection; thereafter, development of the second-generation schizonts takes about 50 h.

At various times during schizont development, homogeneous populations of parasitized intestinal cells were isolated [21]. Purified plasma membranes were obtained from these cells according to a procedure developed by M.A. Fernando and J. Pasternak (unpublished results). Briefly, the infected cells were suspended in Tris/KCl/$MgCl_2$ buffer (0.04 M Tris, 4 mM KCl, 3 mM $MgCl_2$; pH 7.4) at a concentration of $30 \cdot 10^6$ cells/ml. For each homogenization 0.7 ml of this suspension was mixed with 3.3 g of glass beads (0.5 mm diameter) in the disruption vial of a Mickle disintegrator and the cells were disrupted by homogenization for 30 s. The homogenate was recovered by washing the glass beads with 15—20 ml of Tris/KCl/$MgCl_2$ buffer and was then centrifuged at $1000 \times g$ for 15 min to pellet unbroken cells, schizonts and nuclei, and again at $13\,200 \times g$ for 15 min to sediment mitochondria. The resulting supernatant was centrifuged through a discontinuous sucrose gradient in a Beckman SW27 rotor at $80\,900 \times g$ for 16 h. The gradient consisted of 14, 7 and 7 ml of sucrose of densities 1.15, 1.10 and 1.00, respectively, and finally 9 ml of supernatant. The sucrose solutions contained 0.02 M Tris, pH 7.4, as well as 0.05 M Na^+ and K^+ added as chloride salts and 0.005 M $MgSO_4$. Plasma membranes banded as two layers, one at the interface between the sucrose of density 1.00 and the applied supernatant, and the other just below this interface. These membranes were removed from the gradient and washed thoroughly in distilled water adjusted to pH 7.0 before being used for either diffraction analysis or assays. The plasma membranes that accumulated in the uppermost layer were purer than those in the second layer as judged by electron microscopy. The samples from the former layer only were routinely used for the diffraction studies and lipid analyses. However, in order to get enough material for liposome preparation it was necessary to combine these two fractions. This pooling of material is justified because the transition temperatures of the two separate plasma membrane fractions from late-infected cells were the same. Basolateral membranes of epithelial cells from the small intestine were also isolated from uninfected chickens [23].

Schizont isolation. Infected cells that were isolated at 100 h postinfection were treated for 6 min with 0.0001% trypsin (10^6 cells/ml) at 17°C. Trypsin inhibitor was then added at a final concentration of 0.001%. The cells were washed in Tris/KCl/$MgCl_2$ buffer containing 0.25 M sucrose, resuspended at a final concentration of 10^7 cells/ml in the same buffer and homogenized by 3–7 strokes of a Dounce homogenizer using the loose-fitting pestle. This treatment breaks open the cells while the nuclei and schizonts remain intact. Schizonts were separated from the host cell nuclei and unbroken cells by centrifuging the homogenate through a discontinuous sucrose gradient at $19\,600 \times g$ for 15 min in a Sorvall HB4 rotor. The gradient consisted of 10 and 7 ml of sucrose in Tris/KCl/$MgCl_2$ buffer of densities 1.22 and 1.15, respectively, with 2–5 ml of homogenate layered on top. The schizonts banded at the interface between the two sucrose solutions whereas nuclei and unbroken cells banded at higher densities lower in the gradient. The schizonts were removed from the gradient,

washed in Tris/KCl/$MgCl_2$ buffer, resuspended at a final concentration of $3 \cdot 10^7$ cells/ml in fresh buffer and disrupted in a Mickle disintegrator as before. The homogenate was recovered by washing the glass beads with Tris/KCl/$MgCl_2$ buffer and was then centrifuged at 13 200 $\times g$ for 15 min to remove nuclei, mitochondria and unbroken schizonts. The resulting supernatant was centrifuged at 85 000 $\times g$ for 2 h to obtain a microsomal pellet. These microsomal membranes were washed in distilled water, adjusted to pH 7.0, and used for diffraction studies. In a few experiments the microsomal membrane fraction from schizonts was centrifuged through the sucrose gradient used to purify plasma membranes from host cells. The schizont membranes banded at a point further down in the gradient than do the plasma membranes.

X-ray diffraction. Isolated plasma membrane and schizont membranes were prepared for X-ray diffraction as previously described [15,16]. Wide-angle diffraction patterns were recorded from the membranes using CuKα radiation from a point-focused X-ray tube (type PW 2103/01) on a Philips (type 1030) camera under conditions in which the samples retain 50—75% moisture with respect to final dried weight [16]. For each sample, patterns were recorded at 41°C which is the physiological temperature for chickens. The lipid phase transition temperature, designated as the highest temperature at which gel-phase lipid can be detected, was also determined. The specimen to film distance was calibrated using Teflon. Densitometer tracings of the diffraction patterns were made with a Clifford densitometer, model 345.

Lipid analysis. Lipids were extracted from the membranes with chloroform/methanol (2 : 1, v/v) [24]. Fatty acids were obtained by heating the lipid extract in 1 ml 0.1 N methanolic NaOH for 2 h at 95°C under N_2. 1 ml of water was added and the sample was washed three times with 2 ml of pentane. The free fatty acids were recovered by acidifying the methanolic layer with HCl and extracting with 2 ml of pentane. Methyl esters of the fatty acids were prepared by adding 1 ml of 14% BF_3 in methanol and heating the mixture to 95°C for 10 min in a N_2 atmosphere [25]. The esters were extracted with 2 ml of pentane and 1 ml of water, and their purity confirmed by thin-layer chromatography. The methyl esters were separated and identified by flame ionization gas chromatography using a stainless steel column (182.9 × 0.64 cm) packed with 10% EGSS-x in 100/120 Supelcoport and maintained at 180°C. Peak areas were determined with a mechanical disc integrator.

Cholesterol was assayed according to the method of Zak et al. [26]. Phospholipid phosphorus was determined using a modification of Bartlett's procedure [27] as described by Johnston [28]. The lipid extract was treated with concentrated sulphuric acid for 3 h at 250°C and the phosphorus determined using the Fiske-SubbaRow reagent.

Liposome preparation. Liposomes for X-ray diffraction were prepared from the total lipid extracts of the membranes by the procedure of Demel et al. [29]. Approximately 5 mg of the lipid sample were dried onto the sides of a conical reaction vial under N_2. Residual solvent was removed in a vacuum dessicator. The dried lipid was weighed and an equivalent weight of 40 mM Tris/acetate buffer, pH 7.0, containing 100 mM NaCl was added. The sample was mixed to form liposomes and allowed to equilibrate at room temperature. A portion of the liposome preparation was then transferred to a sealed sample

holder and placed in the temperature-controlled jacket of the X-ray diffraction camera.

Trypan blue exclusion test. Within 5 min of killing the chickens, intestinal scrapings were treated with 0.01% trypan blue in phosphate-buffered saline (0.01 M phosphate, 0.85% NaCl, pH 7.4). Exclusion of the dye from cells was scored by phase contrast microscopy.

Results

Preparations of plasma membrane from infected and uninfected cells, although obtained by different isolation procedures, had similar degrees of purity on the basis of both morphological and biochemical criteria. From electron micrographs, the isolated plasma membrane from infected cells was observed to be largely vesicular with no evidence of contamination by recognizable cytoplasmic organelles (Fig. 1). Membrane fractions from both infected and uninfected cells showed enrichments in ouabain-sensitive ($Na^+ + K^+$)-stimulated Mg^{2+}-dependent ATPase activity that ranged from 20 to 25-fold relative to corresponding homogenates on a specific activity basis. Marker enzymes for mitochondrial membrane (cytochrome oxidase) and endoplasmic reticulum (glucose-6-phosphatase) were not detected in purified membrane preparations from either infected or uninfected cells. Moreover, sialic acid content in the plasma membrane fractions from both infected and uninfected cells was enriched 20—25 fold on a protein basis relative to the initial homogenates (Fernando, M.A. and Pasternak, J., unpublished results). With the purity of the plasma membrane preparation established, the physical organization of the lipid component was examined.

Wide-angle X-ray diffraction patterns recorded at 41°C (physiological temperature) for plasma membrane from uninfected chick intestinal cells featured two broad bands centered at Bragg spacings of 4.6 Å and about 10 Å (Fig. 2A). A very faint sharp reflection was also evident at a Bragg spacing of 4.15 Å. The 10 Å reflection is, in part, due to protein, although the exact source of this reflection has not been fully defined [6,30]. The broad diffuse band at 4.6 Å is a lipid reflection that is the consequence of disordered arrangement of hydrocarbon side chains that characterizes lipid in a fluid or liquid-crystalline state [31]. The sharp ring at 4.15 Å derives from an ordered gel phase of the lipid component in which the hydrocarbon chains are packed in a close hexagonal array [31,32].

Diffraction patterns recorded at 41°C for plasma membrane of chick cells that had been isolated at defined times after infection with *E. necatrix* showed the same three reflections. However, in these instances, the sharp ring at 4.15 Å was much more intense than the same reflection from plasma membrane of uninfected cells (Fig. 2B—D). The simultaneous presence of prominent reflections at 4.15 Å and 4.6 Å in the diffraction patterns from plasma membrane of infected cells indicates that both liquid-crystalline and gel-phase domains are extant. Since the relative intensities of these two X-ray bands are proportional to the amounts of these respective lipid phases in the membrane, it is clear that the plasma membrane of infected cells at 90 h after administration of the parasite (i.e. about 24 h after entry of the parasite into the host cell) contains

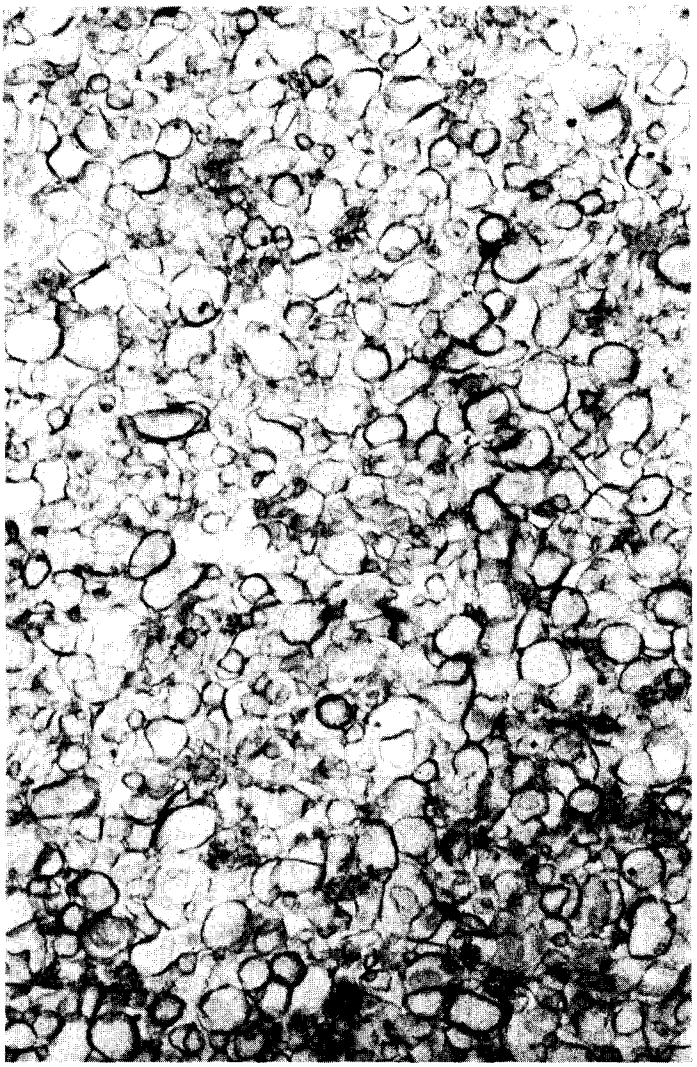

Fig. 1. Transmission electron micrograph of isolated plasma membrane from chick intestinal cells after infection with *E. necatrix*. Membrane preparation is from cells 96 h after infection (×24 300).

significant amounts of gel-phase lipid (Fig. 2B). Earlier stages in the infection process could not be examined because it was not technically feasible to separate adequately uninfected from newly infected intestinal cells. During the second day of schizont development (i.e. from 90 to approx. 100 h after administration of the oocysts) the relative intensity of the X-ray reflection at 4.15 Å increased markedly (Fig. 2C and D), indicating an increased sequestering of membrane lipid into the gel phase. The progressive accumulation of gel-phase lipid in the plasma membrane of the host cell during schizogony is also apparent from densitometric scans of the diffraction patterns (Fig. 3). In the diffraction pattern for membrane from uninfected cells the 4.15 Å X-ray reflection was not of sufficient intensity to register distinctly in the densito-

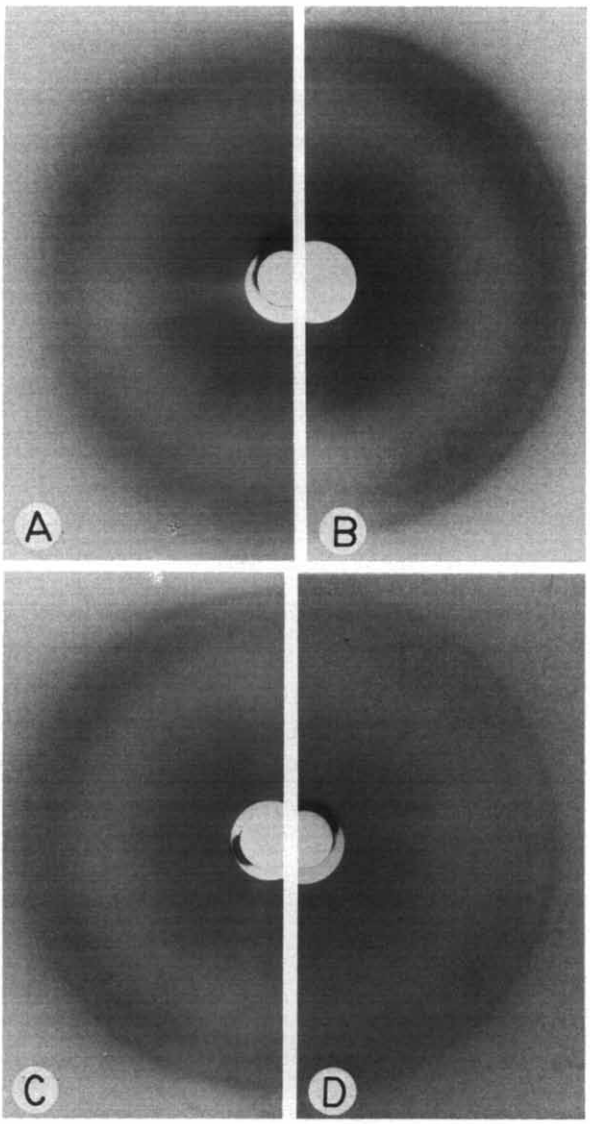

Fig. 2. Wide-angle X-ray diffraction patterns recorded at 41°C. (A) Pattern for plasma membrane from uninfected chick intestinal cells showing (from outside to inside) a very faint sharp band at a Bragg spacing of 4.15 Å and diffuse bands at Bragg spacings at 4.6 Å and about 10 Å. (B—D) Patterns for plasma membranes from infected chick intestinal cells 90, 96 and 100 h, respectively, after infection showing (from outside to inside) a sharp band at a Bragg spacing of 4.15 Å and diffuse bands at Bragg spacings of 4.6 Å and about 10 Å.

metric scans (Fig. 3A), but in the scans of membrane from infected cells a peak at a Bragg spacing of 4.15 Å, which increases in size relative to the 4.6 Å peak with advancing infection, is clearly evident (Fig. 3B—D).

Schizont microsomal membranes were isolated from chick intestinal cells at 96 and 100 h after the initial infection. These schizont membranes contained no gel-phase lipid at 41°C; only the broad 4.6 Å band that denotes liquid-

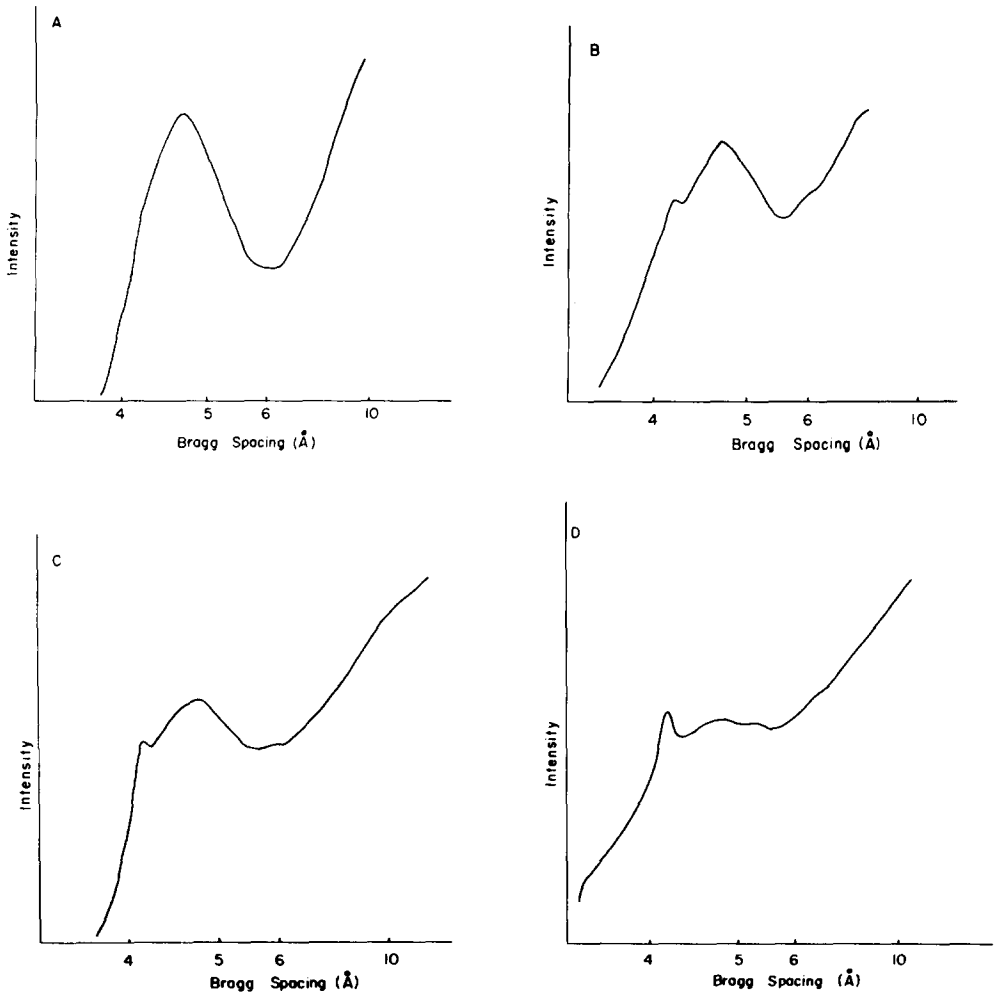

Fig. 3. Densitometric tracings of wide-angle X-ray diffraction patterns for plasma membrane from chick intestinal cells before and after infection with *E. necatrix*. (A) Tracing of a pattern recorded at 41°C for plasma membrane from uninfected cells. (B—D) Tracings of patterns recorded at 41°C for plasma membranes isolated 90, 96 and 100 h, respectively, after infection.

crystalline phase lipid was evident in the diffraction patterns. In addition, diffraction patterns recorded at 41°C from whole schizonts isolated 100 h post-infection showed only the broad diffuse lipid reflection centered at the 4.6 Å Bragg spacing.

The lipid-phase transition temperature of the host cell plasma membrane rises during the development of the second-generation schizonts. Here, the transition temperature is operationally defined as the highest temperature at which the gel-phase lipid can be detected as a reflection at 4.15 Å in the X-ray diffraction pattern. For the plasma membrane from uninfected cells the transition temperature is 43 ± 0.6°C (Table I). This value is slightly above the physiological temperature (41°C) for the chicken. Thus, above 43°C the plasma membrane lipid of uninfected cells is exclusively liquid crystalline; whereas, at

TABLE I

LIPID PHASE TRANSITION TEMPERATURES FOR SCHIZONT MEMBRANES AND HOST CELL PLASMA MEMBRANE AFTER INFECTION OF CHICK INTESTINAL CELLS WITH *E. NECATRIX*

Values represent the highest temperatures at which gel-phase lipid could be detected by wide-angle X-ray diffraction. Standard errors of the means are shown. Numbers in parentheses denote the number of experiments.

Membrane source	Transition temperature (°C)
Plasma membrane from uninfected chick intestinal cells	43 ± 0.6 (3)
Plasma membrane from chick intestinal cells, 90 h postinfection	50 ± 3 (2)
Plasma membrane from chick intestinal cells, 96 h postinfection	51 ± 2 (4)
Plasma membrane from chick intestinal cells, 100 h postinfection	61 ± 2 (4)
Schizont microsomal membranes, 100 h postinfection	40 ± 1 (2)

physiological temperature small amounts of gel-phase lipid are present. By 90 h postinfection, the transition temperature of the host cell membrane is 50 ± 3°C and 10 h later the value is 61 ± 2°C (Table I). The transition temperature for schizont microsomal membranes from 100 h postinfected cells was 40° ± 1°C (Table I).

The lipid phase transition was thermally reversible for host cell plasma membrane preparations from 0 h, 90 h and 96 h postinfected cells. That is, when the temperature was raised above the point of transition, thereby rendering the lipid exclusively liquid crystalline, gel-phase lipid reappeared once the membranes had been cooled to the transition temperature. By contrast, the transition for plasma membranes from 100-h postinfected cells was not thermally reversible. In this case a temperature in excess of 60°C was required to completely melt gel-phase lipid, and during subsequent cooling the gel phase did not reappear until the temperature was below 35°C. This observation suggests that membrane protein(s) may be contributing to the formation of gel-phase lipid in the host cell plasmalemma during infection. To test this notion further the transition temperatures of liposome preparations from total lipid extracts of the purified membranes were examined. The transition temperatures for liposomes from plasma membrane of cells at 0 h and 100 h postinfection were 37°C and 28°C (n = 2), respectively. Thus, liposomes per se do not form the gel phase that is characteristic of infected cells.

TABLE II

SATURATED TO UNSATURATED FATTY ACID RATIOS IN PLASMA MEMBRANE OF CHICK INTESTINAL CELLS BEFORE AND AFTER INFECTION WITH *E. NECATRIX*

Standard errors of the means are shown. Numbers in parentheses denote the number of experiments.

Membrane source	Ratio
Uninfected cells	2.12 ± 0.44 (5)
Infected cells, 90 h postinfection	0.85 ± 0.13 (2)
Infected cells, 96 h postinfection	0.93 ± 0.17 (2)
Infected cells, 100 h postinfection	1.53 ± 0.56 (4)

TABLE III

CHOLESTEROL TO PHOSPHOLIPID RATIOS IN PLASMA MEMBRANE OF CHICK INTESTINAL CELLS BEFORE AND AFTER INFECTION WITH *E. NECATRIX*

Standard errors of the means are shown. Numbers in parentheses denote the number of experiments.

Membrane source	μmol cholesterol/μmol phospholipid
Uninfected cells	0.85 ± 0.14 (5)
Infected cells, 90 h postinfection	0.85 ± 0.13 (4)
Infected cells, 96 h postinfection	0.78 (1)
Infected cells, 100 h postinfection	0.82 ± 0.18 (4)

Fatty acid analyses indicated that the formulation of gel-phase lipid in the plasma membrane of infected and uninfected cells is not attributable to an increase in saturation (Table II; $F = 1.31$; $df = 3,9$; $P > 0.25$). There is a tendency towards higher saturation as infection progresses. For example, the ratio of saturated to unsaturated fatty acids rises from a mean value of 0.85 for membrane from 90-h postinfected cells to 0.93 and 1.53 for membrane from 96-h and 100-h postinfected cells. However, the ratio for membrane from uninfected cells is 2.12 and this is not significantly different from the 100-h postinfected cells (Student's t-test; $t = 0.835$, $df = 7$; $P > 0.30$). Nor was there any significant change in the cholesterol : phospholipid ratio of host cell plasma membrane during the infection period (Table III; $F = 0.027$; $df = 3,10$; $P > 0.75$).

The 'trypan blue exclusion test' revealed that as the proportion of gel-phase lipid in the host cell membrane increases, infected cells that were isolated at 100 h postinfection are not capable of excluding the dye. By contrast, greater than 95% of the uninfected cells do not take up the stain. This observation indicates that alterations in membrane permeability accompany the infection process.

Discussion

It is of concern to this study that the host cell plasma membrane preparations be highly purified. By several criteria, this end was met. For example, (i) ouabain-sensitive ($Na^+ + K^+$)-stimulated Mg^{2+}-dependent ATPase activity and sialic acid content, both well recognized markers for plasma membrane, were enriched 20—25-fold relative to homogenates; (ii) transmission electron microscopy confirmed that the plasma membrane fraction was free of contaminating cytoplasmic organelles, and (iii) there were no detectable cytochrome oxidase and glucose-6-phosphatase activities in the purified preparations indicating that mitochondrial and microsomal membranes were not being isolated with the plasma membranes of the host cells to any significant extent. Indeed, the levels of enrichment of cell surface markers in the isolated intestinal cell plasma membrane fraction noted here were similar to those found by Fujita et al. [33] who isolated plasma membrane from rat intestinal mucosa by a similar technique. Moreover, it is clear that the gel-phase lipid in plasma membrane fractions isolated from infected cells is not attributable to cross-contamination by para-

site membranes since the transition temperature for microsomal membranes from schizonts, that were collected from 100-h postinfected cells, is 21°C less than that for corresponding host cell plasma membrane. A microsomal fraction is, by nature, heterogeneous and could be expected to contain vesicles of membrane derived from several organelles of the schizont including plasma membrane. In addition, diffraction patterns recorded at 41°C for whole schizonts collected from 100-h postinfected cells showed only liquid-crystalline lipid.

The acquisition of gel-phase lipid in membranes at physiological temperature has been documented in various plant tissues that are undergoing deterioration during senescence [15—17]. As senescence intensifies in these tissues, the accumulation of gel-phase membrane lipid is accompanied by an increase in the transition temperature to a point well above physiological temperature. The phase change in these membranes first appears during the early stages of senescence but becomes increasingly apparent during late senescence when cellular autolysis and a decline in membrane biochemical activity have reached an advanced stage [16]. A similar phenomenon seems to occur during pathogenesis in chick intestinal cells following coccidial infection. Between 90 and 100 h after the administration of *E. necatrix* oocysts, substantial proportions of the host cell plasmalemma acquire gel-phase lipid.

It is difficult to define clearly the biological state of the host cell during the later stages of schizogony. During this time period, i.e. from 90 to 100 h postinfection, the host cell surface continues to increase, extra rounds of host nuclear DNA replication that were induced by the parasite cease and nucleolar enlargement persists. Notwithstanding these diverse cellular processes, the imminent fate of an infected cell is deterioration. By 115 h postinfection, the host cell cytoplasm is an advanced state of autolysis and by 120 h the cells lyse. The phase properties of the host cell plasma membrane cannot be monitored during the final 20 h period (i.e. from 100 to 120 h postinfection) because it is not possible to purify cells in this advanced stage of infection. In addition it is not possible to separate infected cells from uninfected cells prior to 90 h postinfection. Thus, the onset of the change in the host cell plasma membrane could not be delineated. However, it should be noted that the induced phase change in host cell plasma membrane is highly selective in the sense that the lipid of schizont membranes remains exclusively liquid crystalline.

The formation of crystalline lipid in the plasma membrane of infected cells is not due to any change in relative sterol content. Nor does it reflect an increase in fatty acid saturation for there is no significant difference between saturated to unsaturated fatty acid ratios for plasma membranes from uninfected and 100-h postinfected cells. The apparent drop in this ratio between membranes of uninfected cells and cells 90 h after infection reflects a tendency towards higher unsaturation, but in spite of this the lipid phase transition temperature, as measured by X-ray diffraction, rises during this period. It is possible that the unique phase properties of the host cell plasmalemma are, in part, attributable to modulation of one or more membrane proteins during schizogony. At least three observations support this notion. First, the irreversibility of the thermotropic transition for membranes from 100-h postinfected cells may be the consequence of protein denaturation. Second, the inability of liposomes from the plasma membrane of 100-h postinfected cells to assume the kind of crystal-

line array found in the membranes may be due to the absence of specific proteins. Moreover, in contrast to uninfected cells, the infected ones are refractory to mechanical breakdown and very sensitive to trypsin-mediated lysis [20]. The latter physical property indicates that the protein organization of the host cell plasma membrane has been altered as a result of the infection. However, it is also conceivable that either heating to 60°C or the steps that are required for liposome formation cause extensive rearrangement of the lipid molecules such that regrouping into gel-phase domains is no longer possible.

The changed physical state of the host cell plasmalemma following infection may lead to partial loss of membrane functions. Not only would the gel-phase regions of infected cell membranes be devoid of enzyme activity [32] but such regions could also alter the permeability properties of the membrane [34]. In model systems, the ionic permeability of liposomes containing pure phospholipids increases at the phase transition temperature [34—36]. This leakiness has been attributed to the inability of gel-phase lipid to form a smooth interface with adjacent fluid-lipid regions [34]. Extensive gel-phase lipid in the plasma membrane of coccidia-infected cells could account for the change in permeability that enables these cells to take up trypan blue. During the infective cycle of cytocidal viruses, host cells also become stainable with trypan blue [37]. Carrasco [38,39] has proposed that this leakiness is induced by specific viral components in the host cell plasmalemma. Since viral maturation leads to host cell deterioration, the leakiness of virally infected cells may reflect the formation of increasing proportions of gel-phase lipid in the plasmalemma as is the case with coccidia-infected cells. In this context, it is perhaps noteworthy that an increase in the microviscosity of the host plasma membrane during viral infection has been detected by spin-labelling procedures [40,41].

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