Phospholipid Synthesis in HeLa Cells Exposed to Immunoglobulin G and Complement

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1. HeLa cells were cultured in the presence of heterologous immunoglobulin G and guinea-pig serum together with [32 P]phosphate. 2. Incorporation of [32 P]phosphate was significantly stimulated by anti-HeLa immunoglobulin G and complement-sufficient serum compared with immunoglobulin G from unimmunized rabbits and complement. Within 2.5 h heat-inactivated guinea-pig serum and anti-HeLa immunoglobulin G stimulated [32 P]phosphate incorporation to the same extent as heat-inactivated complement and immunoglobulin G from unimmunized rabbits. 3. Compared with cells exposed to immunoglobulin G from unimmunized rabbits together with complement, anti-HeLa immunoglobulin G with complement increased the phospholipid content of HeLa cells twofold within 5 h of incubation. 4. Exposure of HeLa cells to anti-HeLa immunoglobulin G and complement for 5–22 h resulted in a twofold increase in the net accumulation of [32 P]phosphate in sphingomyelin and phosphatidylcholine and a 50% increase in the net accumulation of [32 P]phosphate in phosphatidylethanolamine, compared with cultures exposed to immunoglobulin G from unimmunized rabbits and complement. 5. A transient accumulation of 32 P-labelled lysophosphoglycerides in HeLa cells exposed to antibody and complement was detected, confirming previous findings (Güttler & Clausen, 1969b). 6. The stimulation of [32 P]phosphate turnover occurred in cells filling up their cytoplasm with vacuoles. This supports the suggestion that the accumulation of phospholipid in these cells may be concerned with the synthesis and function of cytomembranes.

Exposure of HeLa cells to IgG† and complement-sufficient guinea-pig serum has been shown to increase the cellular content of phospholipid and cholesterol (Güttler & Clausen, 1969b). It was observed that the increase in lipids was significantly higher in cells exposed to anti-HeLa IgG and complement compared with cells exposed to control IgG and complement. Electron microscopy of cells exposed to anti-HeLa IgG and complement revealed ruffling and thickening of the plasma membrane and a marked increase in vacuoles, multivesicular bodies and dense granules (Güttler & Vive Larsen, 1970). These morphological changes of the plasma membrane and the formation of vacuoles may account for the observed increase in phospholipid and cholesterol in the damaged cells (Bensch *et al.*, 1961; Gordon, 1969). Bensch *et al.* (1961) have reported that lipoproteins are taken up by cells from the growth medium to repair cellular damage. It is now clear that phospholipids can exchange between different intracellular organelles (McMurray & Dawson, 1969; Jungalwala & Dawson, 1970; Blok *et al.*, 1971), between cultured

cells (Peterson & Rubin, 1970) and between cells and the serum lipoproteins present in their growth medium (Peterson & Rubin, 1969).

The present experiments were carried out to examine the synthesis and turnover of total and individual phospholipids in HeLa cells exposed to IgG and complement (Güttler & Clausen, 1969b). The results reveal that phospholipid metabolism in HeLa cells is stimulated by exposure to anti-HeLa IgG and complement compared with cells exposed to control IgG and complement.

Experimental

Chemicals

[32 P]Phosphate (sp. radioactivity 0.06–0.10 mCi/mg of P) was obtained as a sterile iso-osmotic solution of sodium orthophosphate in phosphate buffer, pH 6–7, from ISOTOP service, Aktiebolaget Atomenergi, Studsvik, Sweden. Reference mixtures of phospholipids and other chemicals were obtained as previously described (Güttler & Clausen, 1969b).

Cells

Cell culturing. HeLa cells (Gey) (code no. 71–116, Microbiological Associates, Bethesda, Md., U.S.A.)

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† Abbreviation: IgG, immunoglobulin G.

were cultured in a water-saturated atmosphere of $O_2 + CO_2 + N_2$ (25:5:70) as described by Güttler & Clausen (1969a,b).

Cell counting. The cell sheet was scraped off and dislodged flakes were sucked up and down ten times in a 20ml syringe to form a suspension of liberated cells. Duplicate 0.1 ml portions of this cell suspension were withdrawn and incubated for 24h in equal volumes of 0.1 M-citric acid for counting as described by Güttler & Clausen (1969b). In counting more than 1000 cells the mean error may be calculated from Poisson's law (Plum, 1936). With between 1000 and 6000 cells in groups of 137–147 the mean error expressed as a percentage of the counted number of cells per group was found to be 8.5–9.2%.

Immunological procedures

Immunization and isolation of immunoglobulin G. Immunization of animals and isolation of IgG were performed as previously described (Güttler & Clausen, 1969b). The isolated IgG was tested by immunoelectrophoresis and for antibody activity by double immunodiffusion in agar. The anti-HeLa antibody specificity of the IgG fraction was tested by complement-fixation tests on incubation with HeLa antigen and with horse serum, the latter being used in the same concentration as in the medium employed for cell culturing (Volkert *et al.*, 1964). The antibody titre of the pooled IgG was tested by electrophoresis in agarose gel containing the HeLa antigen (modified after Laurell, 1967).

Complement and control rabbit sera. Complement and control rabbit sera were isolated as previously described (Güttler & Clausen, 1969b). The complement titre of the pooled guinea-pig serum was determined as described by Kabat & Mayer (1961).

Incubation conditions

Monolayers of HeLa cultures were used in all experiments. The same batch of medium was used for inoculation and medium change 18h before an experiment (Paul, 1961). At the initiation of an experiment, the medium was sucked off and 25ml of phosphate-free culture medium added. After incubation for 2h, [^{32}P]phosphate ($50\mu Ci/10^6$ cells) was added together with 4ml of fractionated, dialysed and vacuum-concentrated IgG appropriately diluted in respect to antibody titre (cf. Güttler & Clausen, 1969b). The same amount of control IgG (approx. 7mg/ml of culture medium) isolated from non-immunized rabbits was added to control cultures. Complement-sufficient or heat-inactivated guinea-pig serum was added (10%, v/v) to all cultures. At the termination of the exposure to IgG, guinea-pig serum and [^{32}P]phosphate, the medium was poured off and the Roux bottles were immediately placed on

crushed ice. The cell sheet was washed twice in ice-cold Hanks solution and cells were recovered.

Extraction of lipids

Degradation of phospholipids may occur in a long-term (18h) extraction procedure (Hanahan, 1960). Thus procedures for the extraction and purification of phospholipids were briefly examined to study chemical changes that might occur in phospholipids during the isolation procedure. An 18h-extraction procedure 4°C with chloroform-methanol (2:1, v/v) (Güttler & Clausen, 1969b) containing ^{32}P -labelled diacyl phosphoglycerides, followed by evaporation to dryness at 37°C under N_2 (procedure a, Table 1), resulted in radioactivity appearing in lysophosphatidylcholine and lysophosphatidylethanolamine. However, an improved method diminished the deacylation by about 100 times without decreasing the yield of total phospholipid (procedure b, Table 1). This procedure (b) was thus adopted and was as follows. Centrifuged HeLa cells were sonicated for 0.5min at 20kHz and 32–35W (Branson Sonic Power Co., Danbury, Conn., U.S.A.) in 1ml of ice-cold chloroform-methanol (2:1, v/v) at 0°C (the tubes were immersed in ice). After sonication the tubes were filled with N_2 , sealed and kept for 30min at –60°C, followed by 30min at 4°C, twice in succession. Lipid extracts were recovered by centrifugation at 7000g/20min at 4°C and the procedure was repeated. The combined extracts (2.0ml) were centrifuged for 30min at 4°C and 20000g (r_{av} , 7.8cm) and the clear supernatant was evaporated to dryness on a rotary evaporator (Albers & Lowry, 1955) at 4°C. Lipid extracts were immediately redissolved in chloroform-methanol (1:1, v/v) at 4°C and partitioned as described by Güttler & Clausen (1969b).

Phospholipids

Determination of total phospholipid P and total radioactivity in lipid extracts. Four 5 μ l samples were partitioned for determination of lipid P and four 5 μ l samples for determination of radioactivity. Inorganic P was determined after ashing by a micro-adaptation of the Bartlett (1959) method (Güttler & Clausen, 1969b). Radioactivity was determined as described below. The partition technique of Webster & Folch (1961) removed all of the inorganic [^{32}P]phosphate mixed with a crude lipid extract. With the amount of phospholipid usually present (0.8–3 μ g of lipid P) there was no measurable loss of phospholipid to the upper phases.

Separation and analysis of individual phospholipids. Two-dimensional t.l.c. (Güttler & Clausen, 1969b) was used to separate individual phospholipids labelled with [^{32}P]phosphate. After development, plates were exposed to iodine vapour, lipids were

Table 1. *Influence of extraction procedure on diacyl phosphoglycerides*

HeLa cells were grown in the presence of [32 P]phosphate for 72h and the individual phospholipids extracted and separated by t.l.c. The spots containing 32 P-labelled phosphatidylcholine and phosphatidylethanolamine were extracted from the silica gel by chloroform-methanol (2:1, v/v). The chloroform-methanol solution containing [32 P]phosphatidylcholine (56mCi/mmol of P) and [32 P]phosphatidylethanolamine (52mCi/mmol of P) was used as solvent for the extraction of phospholipids from unlabelled HeLa cell cultures. By procedure (a) the cells were homogenized in the solvent and the supernatant was evaporated to dryness at 37°C under N₂. By procedure (b) the cells were sonicated in the solvent at 0°C, and the tubes were then stored under N₂ for 30min at -60°C and for 30min at 4°C, twice in succession; the extracts were evaporated in a centrifuge-evaporator (Albers & Lowry, 1955) at 4°C. A portion of the resolved extract was washed (Webster & Folch, 1961) and assayed for radioactivity and phospholipid P as described in the Experimental section. The individual phospholipids were separated by t.l.c. and assayed for radioactivity and lipid P. The means of four determinations are given, with the ranges in parentheses.

Extraction procedure Phospholipid	10 ⁻³ × Total radioactivity of phospholipids of unlabelled HeLa cells (c.p.m.)		10 ¹⁵ × Phospholipid† (mol/cell)	
	(a)	(b)	(a)	(b)
Total phospholipid	174 (160-188)	108 (103-112)	60.7 (55.7-67.5)	68.9 (65.9-71.9)
Lysophosphatidylcholine	4.7 (2.7-5.6)	0.03 (0.02-0.06)	5.1 (5.0-5.1)	0.2 (0.1-0.3)
Phosphatidylcholine	40 (26-56)	48 (36-59)	17.2 (16.7-18.8)	30.3 (27.5-34.7)
Lysophosphatidylethanolamine	0.8 (0.5-1.1)	0.6 (0.4-0.5)	8.0 (8.3-9.0)	7.1 (6.4-8.1)
Phosphatidylethanolamine	106 (96-126)	50 (47-53)	22.7 (20.3-26.4)	22.3 (18.4-27.5)
Unknown*	76 (33-102)	18 (17-26)		

* This compound gave colour with iodine vapour and ninhydrin.

† The contribution of the diacyl phosphoglycerides added to the solvent is subtracted. Phosphatidylinositol, phosphatidylserine and sphingomyelin were not analysed.

identified by comparison with standards and adsorbent areas sucked directly into 20ml Pyrex digestion tubes (Rouser *et al.*, 1966). Spots containing known amounts of KH₂PO₄ and of phosphatidylcholine as well as blank areas of the adsorbent layer of appropriate sizes were included. The adsorbent contents of the Pyrex tubes were smoothly dispersed on overall lead shieldings (35mm diam.). Radioactivity was measured in a Philips gas-flow detector type PW 4141 (Philips Gloeilampenfabrieken, Eindhoven, The Netherlands) equipped with an ultra-thin window (less than 0.8mg/cm²), an automatic sample changer (PW 4001) and an Addo-X tape printer (PW 4202). The efficiency of detection was 8-12%. Background counts were on average 3.99c.p.m. and were subtracted before calculation of total radioactivity. The radioactivity recovered after t.l.c. as a percentage of the total radioactivity assayed in the washed total lipid extracts before t.l.c. was on average 93%.

Inorganic P was determined on the same samples by using the procedure described above. The recovery, estimated as described for total radioactivity, was on average 97%. There was a linear relationship between radioactivity and content of phospholipid P of individual 32 P-labelled phospholipids extracted from HeLa cells and analysed by t.l.c. (Fig. 1).

Statistical evaluation

All values obtained are the results of duplicate or quadruplicate determinations. The cultures were paired and the same type of guinea-pig serum was added to both antibody-treated and control IgG-treated. The significance of the difference between paired cultures was tested and the difference between pairs exposed to complement-sufficient and to heat-inactivated serum was compared (Croxtton, 1959).

Fig. 1. Total radioactivity as a function of phospholipid phosphorus of individual [^{32}P]phosphate-labelled phospholipids analysed by t.l.c.

Phospholipids of HeLa cells were labelled and extracted by procedure (b) as described in Table 1. The lipid extracts were diluted by appropriate volumes of chloroform-methanol (2:1, v/v) to obtain the amounts of phospholipid P given in the figure. The individual phospholipids were separated by t.l.c. and identified as described by Güttler & Clausen (1969b). The outlined spots were sucked directly into digestion tubes (Güttler & Clausen, 1969b), and the radioactivity and inorganic phosphate were determined as described in the Experimental section. Each point is the average of duplicate determinations. ▽, $10^{-4} \times$ Phosphatidylethanolamine; ▲, $10^{-4} \times$ phosphatidylcholine; Δ, $10^{-2} \times$ lysophosphatidylcholine; □, $2 \times 10^{-3} \times$ sphingomyelin; ○, $5 \times 10^{-4} \times$ (phosphatidylinositol + phosphatidylserine).

Results

Incorporation of [^{32}P]phosphate into total phospholipid

Incubation of HeLa cells with anti-HeLa IgG and complement stimulated [^{32}P]phosphate incorporation into phospholipid more than with control IgG and complement-sufficient serum (significant at the P 0.005 level at 5 h of incubation; Table 2). The stimulation of [^{32}P]phosphate incorporation by antibody and complement was very pronounced after incubation for 22 h. At this point the significance of the difference between paired cultures was 0.001 (Table 2).

Table 2. Incorporation of [^{32}P]phosphate into the total phospholipids of HeLa cells grown in the presence of immunoglobulin G and complement

Incubation conditions	0.5		2.5		5		22	
	cIgG	αIgG	cIgG	αIgG	cIgG	αIgG	cIgG	αIgG
Specific radioactivity per 10^6 cells	4.6 (3.4–5.8)	4.3 (2.1–6.5)	167.6 (138.1–197.1)	185.7 (144.1–227.3)	544.1 (502.4–585.8)	714.7 (641.0–788.4)	2591.3* (2162.8–3019.8)	5116.4* (4526.9–5705.9)
P_{Xp}	69.3 (67.6–71.0)	50.0 (47.1–52.9)	81.8 (70.0–93.6)	88.2 (62.0–114.4)	112.3 (105.1–119.5)	154.9 (149.9–159.9)	121.0* (117.3–124.7)	195.8* (168.9–222.6)
$10^{15} \times$ Phospholipid (mol/cell)	<0.05		<0.1		>0.005		<0.001	
P_{Xp}	<0.05		<0.2		>0.005		>0.005	

* Means of three determinations.

Table 3. Incorporation of [³²P]phosphate into the individual phospholipids of HeLa cells grown in the presence of immunoglobulin G and complement

Symbols and experimental conditions are as described in Table 2. After the incubation the phospholipids were extracted and analysed by t.l.c. as described in the Experimental section. Results are expressed as specific radioactivity (c.p.m./μg of lipid P) in 10⁶ cells. The means of four determinations, with the ranges in parentheses, are given. *P*_x^b indicates the significance of the difference between paired cultures.

Incubation conditions ...	0.5*		2.5		5		22†	
	cIgG	âIgG	cIgG	âIgG	cIgG	âIgG	cIgG	âIgG
Phospholipid								
Phosphatidylethanolamine	3.1 (2.4-3.4)	2.3 (1.8-2.9)	102 (97-108)	127 (105-146)	441 (407-510)	664 (563-752)	2507 (2317-2697)	4979 (4403-5555)
<i>P</i> _x ^b			<0.05		<0.005		<0.025	
Lysophosphatidylethanolamine	8.2†	5.6 (1.8-7.8)	91 (30-166)	171 (130-218)	312 (176-497)	254 (92-439)	500 (503-497)	679 (558-801)
Phosphatidylcholine	2.7 (1.7-4.6)	1.4 (1.3-1.5)	226 (160-298)	291 (219-358)	691 (594-760)	932 (808-1025)	2894 (2866-2922)	6465 (6271-6659)
<i>P</i> _x ^b			<0.1		<0.05		<0.005	
Lysophosphatidylcholine	0.4 (0.0-0.6)	1.3 (0.5-2.5)	5 (1-19)	43 (20-71)	6 (0-13)	121 (47-167)	0 (0-0)	66 (62-69)
<i>P</i> _x ^b			<0.05		<0.05		<0.005	
Sphingomyelin	0.8 (0.3-1.6)	0.5 (0.5-0.5)	36* (19-54)	16 (9-24)	59 (43-73)	97 (74-108)	709 (702-717)	2443 (2304-2582)
<i>P</i> _x ^b					<0.02		<0.01	
Phosphatidylinositol and phosphatidylserine	21.2 (11.6-26.8)	29.1 (11.9-38.5)	240 (159-323)	272 (228-357)	546 (253-741)	1052 (460-1598)	2691 (2458-2925)	3712 (3103-4321)
<i>P</i> _x ^b					<0.05		<0.2	

* Means of three determinations.
† Means of two determinations.

Heat-inactivated guinea-pig serum, together with anti-HeLa IgG or control IgG, stimulated [32 P]-phosphate incorporation within 2.5 h of incubation to the same extent as control IgG and complement-sufficient serum.

The effect of IgG and complement on the accumulation of phospholipid was examined by the direct measurement of inorganic P in the washed lipid extracts. Anti-HeLa IgG and complement stimulated phospholipid synthesis of HeLa cells twofold within 5 h of incubation as compared with cells exposed to control IgG and complement, significant at the 0.005 level (Table 2).

Incorporation of [32 P]phosphate into individual phospholipids

Mean distribution of label. The contribution of phosphatidylinositols+phosphatidylserine to total lipid P was on average 10%, whereas that of phosphatidylcholine was approx. 45%. Within the first 30 min of incubation approx. 50% of the label was present in the phosphatidylinositols+phosphatidylserine area with a higher incorporation in cells exposed to antibody and complement.

Only 20% of the label was present in phosphatidylcholine, whereas the percentage of label present in the phosphatidylethanolamine area at 0.5 h of incubation was in harmony with the contribution of this phospholipid to the pool of total lipid P. In the interval between 2.5 and 22 h of incubation the distribution of the label suggested that the rates of [32 P]phosphate incorporation into phosphatidylcholine and phosphatidylethanolamine were identical. At the end of turnover (22 h) the percentage of label present in the sphingomyelin area was one-third to one-half of the contribution of this phospholipid to the pool of total lipid P.

Specific radioactivity of the individual phospholipids. The earliest alteration of phospholipid metabolism induced by IgG and complement was a stimulation of [32 P]phosphate incorporation into lysophosphatidylcholine detectable after 0.5 h of incubation (Table 3). The stimulation of [32 P]phosphate into lysophosphatidylcholine by anti-HeLa IgG and complement was higher compared with paired cultures exposed to control IgG and complement. This was significant at the 0.05 level at 2.5 h and 5 h of incubation (Table 3), and the specific radioactivity of lysophosphatidylcholine reached a maximum within 5 h of incubation and then declined (Table 3). The transient increase in specific radioactivity of lysophosphoglycerides was most pronounced in cultures exposed to anti-HeLa IgG and complement. Heat-inactivated guinea-pig serum together with anti-HeLa IgG or control IgG stimulated [32 P]phosphate incorporation within 2.5 h of

incubation to the same extent as control IgG and complement-sufficient serum.

Phosphatidylinositol (+phosphatidylserine) and lysophosphatidylethanolamine rapidly became labelled (Table 3). However, the radioactivity in the phosphatidylinositol and the phosphatidylserine areas on t.l.c. sometimes overlapped. In well-separated chromatograms, however, phosphatidylinositol showed a specific radioactivity approx. 10-fold that of phosphatidylserine within the first 5 h of turnover. Anti-HeLa IgG and complement stimulated [32 P]phosphate incorporation into these lipids within 5 h of incubation, significant at the 0.05 level (Table 3). At the end of incubation (22 h) specific radioactivity of phosphatidylinositol and phosphatidylserine was identical regardless of the type of exposure.

In the intermediate stage, from 2.5 to 5 h of exposure, anti-HeLa IgG and complement significantly stimulated the incorporation of [32 P]phosphate into phosphatidylcholine and sphingomyelin respectively (Table 3).

In the interval between 5 and 22 h of incubation the net accumulation of [32 P]phosphate of sphingomyelin and phosphatidylcholine in cells exposed to anti-HeLa IgG and complement was twice that observed in cells exposed to control IgG and complement (Table 3). The net accumulation of [32 P]phosphate in phosphatidylethanolamine was 50% higher in cells exposed to anti-HeLa IgG compared with cells exposed to control IgG and complement. In the same interval, control IgG and complement stimulated [32 P]phosphate incorporation into phosphatidylinositol and phosphatidylserine more than anti-HeLa IgG and complement (Table 3).

Discussion

Synthesis of phospholipids in cultured HeLa cells

The present data on the incorporation of [32 P]phosphate into phospholipids of HeLa cells essentially confirm previous results on the phospholipid turnover of intact cells studied *in vitro* (Tsao & Cornatzer, 1967; McMurray & Dawson, 1969; Weinstein *et al.*, 1969; Pasternak & Bergeron, 1970). In accordance with the work of McMurray & Dawson (1969) each individual phospholipid showed an initial lag period in the incorporation. The lag for [32 P]phosphate incorporation into phosphatidylinositol was less than for the nitrogen-containing phospholipids, probably because the precursor, phosphatidic acid, becomes rapidly labelled (Jungalwala & Dawson, 1970). Pasternak & Bergeron (1970) have recently shown that neoplastic cells contain two pools of phospholipid, one of which is susceptible to enzymic degradation and resynthesis and the other one not. Most of the unstable phospholipid turns over by a process not

involving exchanges of acyl or phosphoryl residues (Pasternak & Bergeron, 1970). If this situation is not unique to the P815Y and HC cells studied by Pasternak & Bergeron (1970) it might be concluded that for HeLa cells that turnover of phospholipid involves the synthesis and breakdown of the entire phospholipid molecule. This suggestion excludes the possibility that the observed increase in [32 P]phosphate turnover of HeLa cells exposed to antibody and complement may be a consequence of increased exchange of phosphoryl units in phospholipids taken up by the cells from the serum lipids of their growth medium. In accordance with observations on neoplastic cells (Pasternak & Bergeron, 1970) the time-course of labelling individual phospholipids of HeLa cells suggests that phosphatidylinositol and part of phosphatidylcholine and phosphatidylethanolamine are relatively easily labelled, with the stable phospholipid being relatively richer in sphingomyelin. In calvaria and bone cell cultures, Dirksen *et al.* (1970) could not demonstrate labelling of sphingomyelin and phosphatidylserine from [32 P]phosphate within 4h of turnover. However, the present study revealed an increase in the specific radioactivity of sphingomyelin within 22h of incubation. The cells contain a sphingomyelin pool comparable with that for the phosphatidylinositols and thus these observations may suggest that the formation of sphingomyelin proceeds relatively slowly (Treble *et al.*, 1970). As pointed out by Pasternak & Friedrichs (1970), extracts of tissues that have been kept for some time undergo chemical degradation. In that case, the percentage of radioactivity found in the phosphatidylcholine area on t.l.c. decreases at the expense of that in the sphingomyelin area. But in contrast with enzymic turnover, such changes are accompanied by the appearance of radioactivity in the lysophosphatidylcholine area and at the origin (Pasternak & Friedrichs, 1970). In the present study the specific radioactivity of lysophosphatidylcholine declined and the radioactivity of sphingomyelin increased at 22h of incubation with IgG and complement. The radioactivity recovered by t.l.c. was approx. 93% throughout the study.

Synthesis of phospholipids of HeLa cells exposed to IgG and complement

The increase in net accumulation of [32 P]phosphate of phosphatidylinositol and phosphatidylserine in the interval 5–22h of exposure to control IgG and complement, as compared with exposure to antibody and complement, is in agreement with the high rate of phagocytic activity (Karnowsky, 1964) revealed by electron microscopy of these cells (Güttler & Vive Larsen, 1970).

Electron microscopy of cells exposed to antibody and complement has revealed gross changes of the

cytoplasmic membrane (Goldberg & Green, 1959; Easton *et al.*, 1962; Benedetti & Emmelot, 1968; Güttler & Vive Larsen, 1970). Moreover, vesicles fuse to vacuoles (Güttler & Vive Larsen, 1970) at the same time as an increase in the activity of unsedimentable lysosomal hydrolases is observed (Güttler, 1971). Observations of Fisher & Mueller (1968), Pasternak & Friedrichs (1970) and Pasternak & Bergeron (1970) lend support to the suggestion (Pasternak & Bergeron, 1970) that membrane turnover, far from indicating cellular inactivity, may be an integral function of cells not specifically concerned with the process of mitosis. Recent observations by Redman (1971) support the suggestion that increased phospholipid metabolism found in leaky erythrocytes is associated with the repair mechanism of the membrane.

The transient increase in [32 P]phosphate turnover of lysophosphoglycerides confirms our previous findings (Güttler & Clausen, 1969b) and lends support to the hypothesis that the diacyl-monoacyl phosphoglyceride cycle (cf. van Deenen, 1965) may operate in these cells during the formation and fusion of vacuoles (Lucy, 1970). The time-course of [32 P]phosphate turnover of lysophosphatidylcholine and of phosphatidylcholine supports the observation of Elsbach *et al.* (1965) of enzymic pathways for the conversion of lysolecithin into lecithin and vice versa.

The twofold net accumulation of [32 P]phosphate of sphingomyelin in cells exposed to antibody and complement compared with cells exposed to control IgG and complement might indicate that the cytomembranes lining the vacuoles that are formed originate from the smooth microsomal membranes (Meldolesi *et al.*, 1971).

The [32 P]phosphate incorporation of the phospholipids that comprise the main part of the lipid moiety of cytomembranes (phosphatidylethanolamine, phosphatidylcholine and sphingomyelin (cf. Dawson, 1966) was appreciably stimulated in HeLa cells exposed to anti-HeLa IgG and complement. The fact that the stimulated phospholipid synthesis occurred in cells filling up their cytoplasm with vacuoles (Güttler & Vive Larsen, 1970), coupled with the fact that the cholesterol/phospholipid ratio remained constant as a function of time (Güttler & Clausen, 1969b), support the suggestion that the observed increase in the cellular content of lipids may be associated with the synthesis and function of cytomembranes (Pasternak & Friedrichs, 1970).

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