Interaction of antibody-bearing small unilamellar liposomes with antigen-coated cells

The effect of antibody and antigen concentration on the liposomal and cell surface respectively

Gregory GREGORIADIS and Anne MEEHAN

Division of Clinical Sciences, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

(Received 27 March 1981/Accepted 20 July 1981)

Human blood lymphocytes were coated with increasing amounts of human kappa chain (2–85 $\mu\text{g}/10^7$ cells) through the linking reagent CrCl_3 . These cells were then exposed to small unilamellar liposomes composed of egg phosphatidylcholine, cholesterol and phosphatidic acid (molar proportions 7:7:1) containing carboxyfluorescein and/or ^{111}In -labelled bleomycin and bearing ^{131}I -labelled affinity chromatography-purified or non-purified anti-(kappa-chain) immunoglobulin G (IgG) [see the preceding paper, Gregoriadis, Meehan & Mah (1981) *Biochem. J.* **200**, 203–210]. In some experiments liposomes contained [^{14}C]phosphatidylcholine. (1) Lymphocytes (10^7) coated with 2–85 μg of kappa chain and exposed to liposomes devoid of IgG or bearing non-purified anti-(kappa chain) IgG bound only a small proportion of the liposomal markers. Even with liposomes bearing the purified anti-(kappa chain) IgG, uptake of the labels improved only slightly for cells coated with up to 10 μg of kappa chain. However, with higher concentrations of the antigen on the cell surface, binding was improved considerably to reach values of 31% (^{111}In -labelled bleomycin) and 43% (^{131}I -labelled IgG) of added liposomes for cells coated with 85 μg of kappa chain. (2) Lymphocytes coated with kappa chain were exposed to liposomes bearing increasing amounts (0–180 $\mu\text{g}/0.9\text{ mg}$ of egg phosphatidylcholine) of purified anti-(kappa chain) IgG. It was found that under the present conditions, binding of all three markers (^{111}In -labelled bleomycin, ^{131}I -labelled IgG and [^{14}C]phosphatidylcholine) was directly proportional to the concentration of IgG on the liposomal surface. However, uptake values remained unchanged above 90 μg of IgG. (3) Antibody-mediated uptake of liposomes by cells coated with the corresponding antigen without loss of their metabolic activities may provide a method of efficient targeting.

Successful targeting of drugs in biological systems via antibody-bearing liposomes is dependent on a number of prerequisites (Gregoriadis, 1980, 1981). These are related to both the liposomal carrier and the target and also to the milieu within which the two interact. For instance, the carrier must be stable as an antibody-bearing drug-containing unit. Furthermore, antigen-recognizing regions of the antibody must be available on the liposomal surface and must be capable of interacting with the corresponding antigens in a given biological milieu without loss of the carrier's stability. We have shown (see the preceding paper, Gregoriadis *et al.*, 1981) that such prerequisites can be satisfied by the use of small

unilamellar cholesterol-rich liposomes incorporating affinity chromatography-purified IgG raised against specific antigens, namely human IgM and kappa chain. Incorporation of IgG in liposomes was carried out either by co-sonication of liposomal lipids in the presence of the immunoglobulin or by interacting the IgG with preformed liposomes bearing antibodies against IgG. Both types of IgG-bearing liposomes were found capable of extensive association with the antigen in the presence of whole blood or blood serum *in vitro* and in the intravascular space of animals *in vivo* while at the same time retaining most of their bilayer stability. However, as targeting in these studies was addressed to free antigens, information obtained is more relevant to free target immunogenic molecules than to similar molecules associated with cell surfaces.

Abbreviations used: IgG, immunoglobulin G; IgM, immunoglobulin M.

Factors expected to determine the degree of interaction between cell surface antigens and antibody-bearing liposomes are likely to include, in addition to those already mentioned, concentration and availability of antibody molecules on the liposomal surface and of antigen molecules on the cell surface (Gregoriadis, 1980). In the present paper we have attempted to study these factors in a model system by interacting small unilamellar liposomes bearing affinity chromatography-purified anti-(kappa chain) IgG with human lymphocytes coated with kappa chain. Our results suggest that, under the conditions employed, optimum association of target cells with liposomes occurs only when antibody and antigen concentrations on liposomal and cellular surfaces respectively attain certain levels.

Materials and methods

The sources and grades of egg phosphatidylcholine, cholesterol, phosphatidic acid, carboxyfluorescein, ^{111}In -labelled bleomycin (5.5 mCi/ μg) and Sepharose CL 6B have been described (Kirby *et al.*, 1980; Gregoriadis & Senior, 1980). Purification of kappa chain (Bence-Jones protein) from the urine of a patient with myelomatosis, production of antiserum in rabbits against the protein and purification of IgG from the antiserum by affinity chromatography are described in detail in the preceding paper (Gregoriadis *et al.*, 1981). [*choline-methyl*- ^{14}C]Phosphatidylcholine (50–60 mCi/mmol) was purchased from New England Nuclear. Labelling of IgG with ^{131}I and of kappa chain with ^{125}I was carried out by the chloramine-T method (Klinman & Taylor, 1969). All other reagents were of analytical grade.

Preparation of liposomes

Small unilamellar negatively charged ^{131}I -labelled IgG-bearing liposomes composed of egg phosphatidylcholine, cholesterol and phosphatidic acid and containing ^{111}In -labelled bleomycin and carboxyfluorescein were prepared as described previously (Gregoriadis *et al.*, 1981) with minor modifications. Briefly, the chloroform in which egg phosphatidylcholine (13.3 μmol), cholesterol and phosphatidic acid (7:7:1 molar proportions) were dissolved was evaporated at 37°C under reduced pressure. The dry film of lipids was then disrupted with 2 ml of 1% (w/v) NaCl in 0.15 M-sodium phosphate buffer, pH 7.4, (phosphate buffer) containing a tracer of ^{111}In -labelled bleomycin [(5–20) $\times 10^6$ c.p.m.], 0.25 M-carboxyfluorescein and, when appropriate, 0.3–5 mg (4 $\times 10^4$ – 10^5 c.p.m.) of ^{131}I -labelled affinity chromatography-purified or non-purified IgG and allowed to stand at 18°C for

1 h. Subsequently, the suspension was sonicated (Kirby *et al.*, 1980) for 5 min and then centrifuged at 100 000 g (r_{av} 8.6 cm) for 30 min. The supernatant was passed through a Sepharose CL 6B column (1 cm $\times$ 25 cm) equilibrated with phosphate buffer to separate entrapped from non-entrapped materials. Liposomes obtained at the end of the void volume were small and unilamellar with an average diameter of 30–60 nm (Kirby *et al.*, 1980). In some experiments [^{14}C]phosphatidylcholine (5 $\times 10^5$ – 10^6 d.p.m.) was incorporated into the lipid phase of liposomes by dissolving the marker together with the other lipids.

Preparation of human blood lymphocytes

The buffy coat from the pooled blood (40–50 ml) of human volunteers was diluted to 90 ml with a buffer composed of 0.8% NaCl/0.02% HCl/0.11% Na_2HPO_4 /0.02% KH_2PO_4 (phosphate-buffered saline A). The mixture was allowed to stand at 18°C for 20 min and the upper layer containing the lymphocytes was then mixed with an equal volume of phosphate-buffered saline A and centrifuged at 1500 g (r_{av} 12 cm) for 8 min in 'Sterilin' 20 ml tubes. The cells were resuspended in phosphate-buffered saline A to 10 ml final volume under which 10 ml of Ficoll/Paque (Pharmacia) was layered carefully. Subsequently the samples were centrifuged at 1500 g for 25 min. Lymphocytes were harvested from the Ficoll-saline interface, washed three times with phosphate-buffered saline A to remove excess Ficoll and suspended in the same buffer to a concentration of 10^7 – 3×10^7 cells/0.1 ml.

Binding of kappa chain to lymphocytes

In a typical experiment, 0.8 ml of 1% (w/v) NaCl, 0.1 ml of lymphocyte suspension containing 10^7 – 3×10^7 cells and 0.1 ml of ^{125}I -labelled kappa chain [0.1 mg and (2–4) $\times 10^4$ c.p.m.] (1.0 ml total volume of sample) were added into each of a series of 3 ml plastic tubes. Binding of kappa chain to cells was then effected with CrCl_3 prepared from a 1% (w/v) stock solution in 1% (w/v) NaCl; dilutions of 1 ml of CrCl_3 with 0.01 M-sodium acetate buffer, pH 5.5, containing 1% (w/v) NaCl were added dropwise to the samples (4 $\times 10^{-4}$ –5 $\times 10^{-2}\%$ final concn. of CrCl_3) which were at the same time vortex-mixed. The samples were allowed to stand at 18°C for 10 min, mixed with 1.0 ml of phosphate-buffered saline A and then centrifuged at 600 g (r_{av} 12 cm) for 8 min. The supernatants were removed and the pellets washed twice with 2 ml of phosphate-buffered saline A. There was no loss of radioactivity from the cells when these were washed further. ^{125}I radioactivity was assayed in portions of the cell pellets suspended in 2 ml of phosphate-buffered saline A and in the supernatants.

Interaction of kappa chain-coated lymphocytes with liposomes

Kappa chain-coated human lymphocytes (10^7 cells) suspended in 1.0 ml of phosphate-buffered saline A were mixed with 0.2 ml (about 1 mg of phospholipid) of various liposomal preparations and incubated at 37°C for 10 min. At the end of the incubation period the samples were mixed with 1.0 ml of phosphate-buffered saline A and immediately centrifuged at $600g$ for 8 min. The pellets were then washed twice with 2 ml of phosphate-buffered saline A. ^{125}I , ^{131}I , ^{111}In and ^{14}C radioactivities were assayed in the suspended cell pellets and supernatants (see below). In one experiment interaction of kappa-coated lymphocytes with liposomes bearing anti-(kappa chain) IgG was carried out in the presence of 0.5 mg of kappa chain.

Assay of carboxyfluorescein

Carboxyfluorescein in appropriately diluted samples of liposomes or media was measured in the absence (free dye) and presence (total dye) of Triton X-100 (1% final concentration) on a Perkin-Elmer 204A fluorimeter, by using excitation and emission wavelengths of 490 and 520 nm respectively. Latent carboxyfluorescein was estimated as the difference in dye values obtained with and without Triton X-100 and expressed as % of total dye in the sample (for details see the preceding paper, Gregoriadis *et al.*, 1981).

Assay of radioactivity

^{111}In , ^{131}I and ^{125}I in liposomes or in cells incubated with liposomes were assayed as described (Gregoriadis & Neerunjun, 1974; Gregoriadis & Senior, 1980). Assay of ^{14}C in the presence of ^{111}In and ^{131}I was carried out after decay of ^{111}In ; interference from ^{131}I was eliminated by setting double isotope counting conditions.

Results and discussion

Studies in the preceding paper (Gregoriadis *et al.*, 1981) showed that IgG can be incorporated (20–40% of the amount used) in small unilamellar cholesterol-rich liposomes prepared by sonication in the presence of immunoglobulin. Incorporated IgG, found localized mostly on the liposomal surface, did not promote bilayer permeability to entrapped solutes, even after interaction with the respective antigen (in phosphate buffer). For liposomes bearing anti-(human IgM) and anti-(human kappa chain) IgG, both purified by affinity chromatography, interaction with the antigens in solution was considerable (e.g. 37% of the kappa chain used). In this report we have attempted to investigate conditions under which liposome-associated antibody can interact with antigen localized on the surface of cells

in spite of possible interference by masking neighbouring macromolecules and by cell (and perhaps liposomal) geometry. In the model system used here, human blood lymphocytes were coated with human kappa chain via CrCl_3 and then exposed to liposomes bearing affinity chromatography-purified anti-(kappa chain) IgG.

Coating of lymphocytes with kappa chain

Fig. 1 shows that binding of kappa chain to lymphocytes in the presence of 4×10^{-4} – $5 \times 10^{-2}\%$ CrCl_3 is sigmoidal. Using 10^7 cells, maximum binding at the highest concentration ($5 \times 10^{-2}\%$) of the linking reagent was 84% of the protein (0.1 mg) added. Only 5% of the protein was linked to the cells at the lowest concentration of the reagent. With increasing number of lymphocytes exposed to the same amount of kappa chain, its binding to cells decreased proportionally (see the legend to Fig. 1) presumably because of a diminished share of protein per cell.

Effect of kappa chain concentration on the surface of lymphocytes on their interaction with antibody-bearing liposomes

Studies on the interaction of kappa chain-coated lymphocytes with liposomes were carried out using 10^7 cells. As already discussed, in the presence of $5 \times 10^{-2}\%$ CrCl_3 this number of cells binds most of the protein used (Fig. 1). Fig. 2 shows the uptake of

Fig. 1. *Binding of kappa chain to lymphocytes*
In a typical experiment human blood lymphocytes (10^7) were exposed to 0.1 mg of ^{125}I -labelled kappa chains [$(2-4) \times 10^6$ c.p.m.] in the presence of increasing concentrations of CrCl_3 . Binding of kappa chains to cells is expressed as a percentage of the amount added. In experiments in which 2×10^7 and 3×10^7 lymphocytes were exposed to kappa chain as above, binding at the highest concentration of CrCl_3 was 40.6 and 29.0% respectively. For other details see the Materials and methods section.

Fig. 2. Interaction of kappa chain-coated lymphocytes with liposomes

Human blood lymphocytes (10^7) coated with increasing amounts of kappa chain ($2\text{--}85\text{ }\mu\text{g}/10^7$ cells) were exposed to liposomes (about 1 mg of phospholipid) composed of egg phosphatidylcholine, cholesterol and phosphatidic acid (7:7:1 molar proportion). Liposomes devoid of IgG ($\blacktriangle$) or bearing non-purified ($\blacksquare$) or affinity chromatography-purified ($\bullet$) ^{131}I -labelled anti-(kappa chain) IgG [(1–2) $\times 10^3$ c.p.m., 60–90 μg] contained a tracer of ^{111}In -labelled bleomycin [(1–5) $\times 10^4$ c.p.m.]. In one experiment lymphocytes were exposed to free anti-(kappa chain) IgG (72 μg) ($\circ$). (a) ^{131}I radioactivity, (b) ^{111}In radioactivity. Results from three to four experiments are expressed as percent ($\pm$ s.d.) of added label bound to 10^7 lymphocytes. In one experiment, after exposure of 10^7 lymphocytes coated with 85 μg of kappa chain to liposomes bearing purified anti-(kappa chain) IgG as above but in the presence of 0.5 mg of free kappa chain, uptake of liposomal ^{111}In and ^{131}I radioactivity by the cells was 5.6 and 3.5% respectively.

liposomal ^{111}In -labelled bleomycin by lymphocytes coated with increasing amounts of kappa chain. With liposomes devoid of IgG, uptake of ^{111}In radioactivity (Fig. 2b) was very low (less than 2% of the amount added) and increased only modestly (to 10%) in cells coated with the maximum amount of kappa chain (85 $\mu\text{g}/10^7$ cells). The presence of non-purified anti-(kappa chain) IgG in liposomes did not improve uptake of the label significantly and the pattern of uptake of the liposomal IgG itself (^{131}I radioactivity, Fig. 2a) was similarly low. Even with liposomes bearing affinity chromatography-purified anti-(kappa chain) IgG, incorporation of liposomal ^{111}In onto cells coated with less than 10 μg of kappa chain was improved only slightly. However, with

higher concentrations of kappa chain ^{111}In uptake increased considerably (to 31%) for cells containing 85 μg of antigen. The pattern of increasing uptake of the marker was paralleled by a similar increase in the uptake of the liposomal anti-(kappa chain) IgG (up to 43%; Fig. 2a) suggesting that association of the liposomal ^{111}In with the cells was mediated by the purified liposomal immunoglobulin. However, judging from the relatively poor (at best, 20%; Fig. 2a) association of purified free anti-(kappa chain) IgG with cells, it is likely that only a portion of the total cell-bound liposomal IgG interacts directly with the antigen, the remainder being carried passively to the cells by its carrier. The possibility that CrCl_3 , available in an active form on the cell surface rather than the kappa chain was responsible for the specific binding of liposomal IgG (and the associated marker) was deemed unlikely in view of the low values obtained both with the control liposomes bearing similar amounts of non-purified IgG and with the anti-(kappa chain) IgG presented to cells in the presence of excess free kappa chain (see legend to Fig. 2). Indeed, involvement of CrCl_3 in non-specific binding was excluded in an experiment in which incubation of kappa chain-coated cells with 1% (w/v) albumin prior to their exposure to liposomes bearing purified anti-(kappa chain) IgG did not alter significantly marker uptake values (results not shown).

Effect of anti-(kappa chain) IgG content of liposomes on their interaction with kappa chain-coated lymphocytes

Having established that purified anti-(kappa chain) IgG can mediate effective association of the contents of its liposomal moiety with cells coated with appropriate amounts of kappa chain (Fig. 2), it was of interest to see whether such association could be influenced by the concentration of the immunoglobulin on the liposomal surface. Therefore, lymphocytes coated with increasing amounts of kappa chain were exposed to carboxyfluorescein-containing liposomes bearing increasing amounts of purified anti-(kappa chain) IgG. In addition to the ^{131}I label carried by the IgG, liposomes were labelled with [^{14}C]phosphatidylcholine in their lipid phase and with ^{111}In -labelled bleomycin in their aqueous phase. Furthermore, the stability of liposomes during interaction with the cells was monitored by measuring the latency of entrapped carboxyfluorescein. It appeared from the results shown in Table 1 that, under the present conditions, uptake of all liposomal radioactive markers by the cells is directly proportional to the concentration of IgG on the liposomal surface for up to about 90 μg of immunoglobulin per liposomal sample added (0.9 mg of egg phosphatidylcholine). Above this amount, uptake

Table 1. *Effect of anti-(kappa chain) IgG content of liposomes on their interaction with kappa chain-coated lymphocytes*
Human blood lymphocytes (10^7) coated with increasing amounts of kappa chain were exposed as described in the legend to Fig. 2 to liposomes (0.9 mg of egg phosphatidylcholine) bearing increasing amounts of ^{131}I -labelled anti-(kappa chain) IgG and containing ^{111}In -labelled bleomycin and carboxyfluorescein in the aqueous phase and ^{14}C phosphatidylcholine $[(4-5) \times 10^4 \text{ d.p.m.}]$ in the lipid phase. Carboxyfluorescein latency in the media after interaction of liposomes (all experiments) with cells was 89.5–91.8%. Latency of carboxyfluorescein in liposomes before their use was $>94.5\%$.

IgG in liposomes (μg)	Kappa chain on lymphocytes (μg)	Binding to 10^7 cells (% of added)		
		^{131}I -labelled IgG	^{111}In -labelled bleomycin	^{14}C Phosphatidylcholine
0	45	—	1.5	5.5
9	45	8.3	2.8	8.8
	63	11.9	3.6	20.9
	76	34.7	15.1	25.4
	45	12.0	2.8	11.9
27	63	17.0	3.5	15.7
	76	47.6	15.2	37.4
90	45	16.4	3.4	12.6
	63	28.1	6.2	21.4
	76	60.9	25.8	44.5
180	45	14.5	2.9	11.5
	63	31.2	8.1	24.4
	76	62.0	25.0	46.1

values remained unchanged. The pattern of uptake of three labels was similar for all cell preparations regardless of their kappa chain content, but uptake values were highest (for all concentrations of liposomal IgG) with cells coated with the highest amount of the antigen (Table 1). Interestingly, judging from the carboxyfluorescein latency values (89.5–91.8%) in the media, loss of liposomal stability in the presence of cells was only minimal (see the legend to Table 1).

In conclusion, our model studies show that both purity and concentration of antibody on the liposomal surface, as well as concentration of antigen on the cell surface, are instrumental for efficient targeting. In the present work, three liposomal markers representing the antibody (^{131}I), the lipid phase of liposomes (^{14}C phosphatidylcholine) and the aqueous phase (^{111}In -labelled bleomycin) were used. With regard to liposomes bearing purified IgG, the concurrent increase in uptake values for all markers (Fig. 2 and Table 1) is compatible with IgG-mediated association of the whole drug-containing liposome–antibody unit with the cells. However, uptake values differed according to the marker and under optimal conditions these ranged from 25.8% (^{111}In) to 60.9% (^{131}I) of the quantity added with ^{14}C showing an intermediate value of 44.5% (Table 1). Such varied behaviour of the markers is not surprising since, for instance, ^{111}In -labelled bleomycin would be expected (Kirby *et al.*, 1980) to leak even from liposomes that have been slightly destabilized through contact with cells

(Pagano & Weinstein, 1978). As it is conceivable that bilayer destabilization may also lead to some loss of phospholipid from the carrier (Kirby & Gregoriadis, 1980), it is likely that values of liposomal IgG uptake (43 and 60.9%, Fig. 2 and Table 1 respectively) more faithfully reflect true liposomal binding to cells. On this assumption, it would appear, under the present conditions, either that the liposome-binding capacity of the number of cells used is somewhat limited, or that only a fraction (albeit a major one) of the liposomes used exhibit affinity for the antigen-coated cells. However, the former possibility can be excluded, since after exposure of the same number of cells to one-tenth of the quantity of liposomes used in Fig. 2 and Table 1 percentage uptake values for all markers remained practically unchanged (results not shown).

It, therefore, seems that IgG is distributed on the liposomal surface in a way that does not endow targeting capacity to the total number of vesicles. Indeed, it may be that a critical number of antibody molecules on the surface of liposomes is required for successful association with cells and some of the vesicles may thus be antibody 'deficient'. This is supported by our previous finding (Gregoriadis & Neerunjun, 1975) showing abolition of targeting in antibody-bearing liposomes that were harvested from the media after exposure to target cells and then presented again to a similar number of unused cells. Such partial targeting capacity of liposomes is unlikely to relate to our method of IgG incorporation onto liposomes or to their size. For

instance, Leserman *et al.* (1980a,b) and Huang *et al.* (1980), using alternative techniques to link antibody onto liposomes of varying sizes, reported limited uptake. Even with the use of monoclonal antibodies, uptake of markers by cells carrying the respective antigens was 13% (Huang *et al.*, 1980) and 10% (Leserman *et al.*, 1980a,b). Interestingly, in another attempt at targeting (Heath *et al.*, 1980) in which uptake of liposomal markers was elevated (60–70%), the F(ab')₂ used for incorporation onto liposomes was derived from non-purified anti-erythrocyte IgG. Surprisingly, however, uptake values were decreased to about 10% when a smaller number of cells (erythrocytes) was exposed to a proportionally smaller number of liposomes. The significance of such findings (in terms of methods of antibody incorporation and its state of purity) or, indeed, their application to other cell types, remains unclear.

Our studies, in conjunction with work already published (Gregoriadis & Neerunjun, 1975; Gregoriadis *et al.*, 1977; Leserman *et al.*, 1980b; Huang *et al.*, 1980; Heath *et al.*, 1980), suggest that successful targeting may be less critically dependent on the method of antibody incorporation onto liposomes or the type of specific antibody used (affinity chromatography-purified or monoclonal) and more on the concentration and mutual accessibility of the reacting moieties on liposomes and cells. This is especially relevant to the state of the antigen, which could become a decisive factor in selecting types of cells as candidates for targeting. However, as our present data suggest, cells can be modified so as to exhibit considerable affinity for liposomes bearing appropriate antibodies. It would, therefore, be of interest to see whether this approach to targeting via surface antigen manipulation (not necessarily by the method used here) could be applied to cells in culture without loss of their metabolic activity. With regard to targeting *in vivo*, an important parameter to be considered is effective contact of liposomes with cells (Gregoriadis, 1980).

To that end there will be a need for extended circulation of the carrier in a stable form in the blood and, when applicable, transcapillary passage. Small unilamellar liposomes made from appropriately selected lipid components (Kirby *et al.*, 1980; Kirby & Gregoriadis, 1980; Gregoriadis & Senior, 1980) should satisfy both conditions.

We thank Mrs. M. Moriarty for excellent secretarial work.

References

- Gregoriadis, G. (1980) in *Liposomes in Biological Systems* (Gregoriadis, G. & Allison, A. C., eds.), pp. 25–86, John Wiley and Sons, Chichester, New York, Brisbane and Toronto
- Gregoriadis, G. (1981) *Lancet* **ii**, 241–247
- Gregoriadis, G. & Neerunjun, E. D. (1974) *Eur. J. Biochem.* **47**, 179–185
- Gregoriadis, G. & Neerunjun, E. D. (1975) *Biochem. Biophys. Res. Commun.* **65**, 537–544
- Gregoriadis, G. & Senior, J. (1980) *FEBS Lett.* **119**, 43–46
- Gregoriadis, G., Neerunjun, E. D. & Hunt, R. (1977) *Life Sci.* **21**, 357–369
- Gregoriadis, G., Meehan, A. & Mah, M. M. (1981) *Biochem. J.* **200**, 203–210
- Heath, T. D., Fraley, R. T. & Papahadjopoulos, D. (1980) *Science* **210**, 539–541
- Huang, A., Huang, L. & Kennel, S. J. (1980) *J. Biol. Chem.* **255**, 8015–8018
- Kirby, C. & Gregoriadis, G. (1980) *Life Sci.* **27**, 2223–2230
- Kirby, C., Clarke, J. & Gregoriadis, G. (1980) *Biochem. J.* **186**, 591–598
- Klinman, N. R. & Taylor, R. B. (1969) *Clin. Exp. Immunol.* **4**, 473–487
- Leserman, L. D., Barbet, J., Kourilsky, F. & Weinstein, J. N. (1980a) *Nature (London)* **288**, 602–604
- Leserman, L. D., Weinstein, J. N., Blumenthal, R. & Terry, W. D. (1980b) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4089–4093
- Pagano, R. E. & Weinstein, J. N. (1978) *Annu. Rev. Biophys. Bioeng.* **7**, 435–468