PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 326–333 (1982)

Amphotericin B: Stimulation of Bone Resorption in Organ Culture[1] (41352)

LUKA RABADJIJA AND PAUL GOLDHABER

Harvard School of Dental Medicine, Boston, Massachusetts 02115

Abstract. Organ culture studies of 5-day-old mouse calvaria show that amphotericin B, a polyene antibiotic, is a potent bone resorption-stimulating agent, as measured by calcium release into the medium, residual calcium, and hydroxyproline in the calvaria at the end of the 7-day culture period, and by histological examination. Nystatin and filipin, two other polyene antibiotics, also induce bone resorption in the same system. Amphotericin B and nystatin are thought to act on natural and artificial membrane systems by binding to cholesterol or other steroids thereby forming aqueous pores that permit the passage of ions and some other solutes. Such alteration of cell membrane permeability may be a crucial initial step in the chain of events leading to bone resorption by these two agents. Inhibition of polyene antibiotic-stimulated bone resorption by indomethacin and dexamethasone suggest that polyene antibiotic-stimulated resorption is mediated by prostaglandin biosynthesis.

Many specific cellular responses, such as contraction, secretion, and fusion, are affected by ionophores, often mimicking the function of more specific agents. This applies particularly to the divalent cation ionophore A23187 (1–6). Recent studies have shown that this ionophore can also stimulate bone resorption in tissue culture (7–10). These observations prompted us to study the effect on bone in tissue culture of some other compounds affecting the transport of ions across cell membranes, such as the polyene antibiotic amphotericin B. We report here that amphotericin B is a potent bone resorption-stimulating factor in tissue culture, as determined by calcium release into the medium, residual calcium, and hydroxyproline in the cultured bones at the end of the experiment, and by histological examination. Two other polyene antibiotics, nystatin and filipin, also induced bone resorption but to a lesser extent.

Material and Methods. Bone cultures were prepared as follows: Calvaria of 5-day-old mice (Swiss albino, Webster strain) were dissected aseptically and mounted individually (concave side down) on stainless steel grids in small plastic petri dishes (35 × 10 mm Falcon No. 3001). Two milliliters of Dulbecco's modified eagle's medium, containing 4.5 mg/ml glucose (M.A. Bioproducts, Walkersville, Md.) and supplemented with 2 ml/100 ml of 200 m*M* glutamine (M.A. Bioproducts, Walkersville, Md.), 5 mg/ml bovine serum albumin, fraction V (Sigma Chem. Co., St. Louis, Mo.), 10 u/ml heparin (E. Lilly and Co., Indianapolis, Ind.), 100 u/ml penicillin, and 100 μg/ml streptomycin were added to each culture. In addition, test substances were included in the media added to the experimental groups, as indicated under Results.

Amphotericin B, nystatin, and filipin

[1] Supported in part by U.S. Public Health Grant DE 02849, National Institute of Dental Research. The authors are grateful for the technical assistance of Ms. Joan Jennings, Lorraine Stevens, Olita Treimanis, and Laura Murphy. Amphotericin B was either purchased from Calbiochem–Behring Corporation, La Jolla, California, or kindly supplied by the Squibb Institute for Medical Research, Princeton, New Jersey. Nystatin (Squibb and Sons, New York, N.Y.) was either purchased from a local pharmaceutical distributor (Mycostatin, sterile powder, 500,000 units/vial) or from Sigma Chemical Company, St. Louis, Missouri. Filipin (U-5956, crystalline complex, 66% pure) was kindly provided by Dr. Whitefield (Upjohn Co., Kalamazoo, Mich.). Parathyroid extract (E. Lilly and Co., Indianapolis, Ind.) was purchased from a local pharmaceutical distributor. Prostaglandin E_2 was a generous gift from Dr. Pike (Upjohn Co., Kalamazoo, Mich.). The following abbreviations have been used: PTE, parathyroid extract; PTH, parathyroid hormone; PGE_2, prostaglandin E_2; DMSO, dimethyl sulfoxide.

0037-9727/82/030326-08$01.00/0

were dissolved in DMSO. The amount of DMSO in the medium never exceeded 0.1% except in one experiment in which 0.2% was used. Indomethacin was dissolved in absolute ethanol and PGE_2 in 70% ethanol. The amount of ethanol also never exceeded 0.1%. These concentrations of DMSO or ethanol per se did not influence the extent of bone resorption when added to non-treated cultures or cultures treated with parathyroid extract (PTE). Bone cultures were placed in a water-jacketed CO_2 incubator (National Appliance Co., Portland, Ore.) at 37°, in a closed and constantly moisturized compartment and flushed for 30 min with a gas mixture consisting of 5% CO_2, 50% O_2, and 45% N_2. In most experiments, there were at least four calvaria per group; in some experiments, there were six, seven, or eight calvaria per group. During the 7-day culture period, the medium was changed twice (every 2–3 days) and the compartment was regassed with the same gas mixture. Used media were analyzed for the total calcium content, using an automated fluorometer (Corning calcium analyzer, Model 940). Appropriate corrections were made for calcium initially present in the various batches of freshly prepared media. Net calcium values were accumulated and subjected routinely to an analysis of variance. The amount of calcium released into the medium above the amount present in fresh medium (net calcium) served as a chemical index for expressing the amount of bone resorption. At the end of the experiment, calvaria were routinely fixed and processed for histological examination using hematoxylin and eosin staining. In some experiments, two to four calvaria from each group were hydrolyzed in 6 *N* HCl at 110°, for 18 hr. Hydrolysates were then analyzed for the amount of hydroxyproline as an index of residual bone collagen, according to the method of Woessner (11) and, also, for the amount of residual calcium, using the Corning calcium analyzer. These data were also subjected to an analysis of variance and used as additional parameters to express the degree of bone resorption.

Results. Table I presents the data from four representative experiments illustrating the effect of amphotericin B on the total amount of calcium released into the medium above the amount present in fresh medium during the 7-day culture period. It may be seen that the addition of 0.1 u/ml PTE gives rise to a substantial increase in calcium released into the medium compared to the untreated controls. In comparison, at a concentration of 5 μg/ml, am-

TABLE I. EFFECT OF AMPHOTERICIN B ON 7-DAY CUMULATIVE CALCIUM RELEASE INTO THE CULTURE MEDIUM[a]

Experimental groups	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Control	0.7 ± 0.62	1.3 ± 0.70	1.7 ± 0.73	0.1 ± 0.74
Parathyroid extract (0.1 u/ml)	8.3 ± 0.62***	9.5 ± 0.99***	6.7 ± 0.73***	8.3 ± 0.74***
Amphotericin (μg/ml)				
100	—	—	—	14.3 ± 0.74***
75	—	—	—	15.3 ± 0.74***
50	—	17.7 ± 0.70***	15.3 ± 0.73***	14.5 ± 0.74***
20	18.2 ± 0.62***	—	—	—
10	16.3 ± 0.62***	17.5 ± 0.70***	15.9 ± 0.73***	—
5	12.9 ± 0.62***	8.9 ± 0.70***	10.3 ± 0.73***	8.7 ± 0.74***
1	3.5 ± 0.62**	—	2.3 ± 0.73	—
0.5	—	2.0 ± 0.81	2.5 ± 0.73	—
0.1	0.1 ± 0.62	—	—	—

[a] Values represent mg/dl (mean ± SEM).
* $P < 0.05$ as compared to the untreated control.
** $P < 0.01$ as compared to the untreated control.
*** $P < 0.001$ as compared to the untreated control.

photericin B caused an increase in calcium release equal to or greater than that obtained with 0.1 u/ml PTE. At a concentration of 10 μg/ml, amphotericin B brought about a large calcium release, approximately double that obtained with PTE. Although no significant additional increase was obtained at higher concentrations of amphotericin B, it should be noted that even at 100 μg/ml, large amounts of calcium were released into the medium. The addition of 1 μg/ml of amphotericin B or less did not consistently increase calcium release over the untreated controls.

Data presented in Table II illustrate the effect of amphotericin B on the amount of calcium and hydroxyproline found in the calvaria at the end of the 7-day culture period. The data are obtained from the same experiments numbered 1, 2, and 3 in Table I. It may be seen that, similarly to the PTE-treated cultures, amphotericin B-treated cultures contained significantly less calcium and hydroxyproline at the end of the experiment as compared to the untreated controls. Both parameters complement the data presented in Table I in illustrating the bone resorption stimulating effect of amphotericin B.

Histological examination of bone sections stained with hematoxylin and eosin revealed that cultures treated with amphotericin B had numerous osteoclasts, many with giant vacuoles ("bubble" osteoclasts), a finding very similar to that observed previously with PTE during rapid bone resorption in culture (12).

In an attempt to unravel the mode of action of amphotericin B, the effect of indomethacin, an inhibitor of prostaglandin biosynthesis (13), was tested in conjunction with amphotericin B. Indomethacin does not inhibit bone resorption induced in tissue culture by PTH (14, 15) or PGE_2 (16) but does inhibit the effect of several other bone resorption-stimulating agents, such as the unidentified bone resorption-stimulating factor(s) present in conditioned medium from cultures of inflamed human gingival tissue (14), dibutyryl cyclic AMP, 8-bromo cyclic AMP, theophylline, 3-isobutyl-1-methylxanthine, 25-hydroxycholecalciferol (17), tumor-promoting phorbol diesters, and mellitin (18). Also, the effect of dexamethasone on amphotericin B-stimulated bone resorption was tested, because this compound and some other glucocorticoids have been shown to inhibit in tissue culture the effect of PTH (19). As shown in Table III, whereas indomethacin (100 ng/ml) had no effect on PGE_2-stimulated bone resorption (100 ng/ml), it did abolish the bone resorption-stimulating effect of amphotericin B (5 μg/ml). At the same concentration, indomethacin only partially but significantly inhibited bone resorption induced by higher concentrations of amphotericin B (data not shown). As may be seen in Table IV, dexamethasone (10^{-8} *M*) also abolished the bone resorption-stimulating effect of amphotericin B (5μg/ml). At 10^{-9} *M*, dexamethasone was not as effective but did reduce the resorption induced by 5 μg/ml of amphotericin B by approximately 50%. As with indomethacin, dexamethasone only partially but significantly inhibited bone resorption induced by higher concentrations of amphotericin B (data not shown).

Results obtained with amphotericin B prompted us to study the effect of two other polyene antibiotics, i.e., nystatin and filipin, in the same system. As shown in Table V, nystatin (10 and 20 μg/ml) also promoted bone resorption, but was less potent as compared to amphotericin B. As also shown in Table V, whereas it had no effect on PGE_2-stimulated bone resorption, indomethacin abolished the bone resorption-stimulating effect of nystatin observed at the concentrations indicated. It should be noted that, similar to amphotericin B, nystatin induced bone resorption even at higher concentrations (50 μg/ml), although the bone resorption-stimulating effect at this concentration was only slightly higher than that obtained with 20 μg/ml (data not shown). As shown in Table VI, at the concentration indicated (3.5 μg/ml), filipin also caused a significant bone resorption-stimulating effect which was abolished by indomethacin. At higher concentrations (14 and 35 μg/ml), filipin was toxic, as determined by histological examination of sections prepared from cultured bones.

Discussion. In the present study, amphotericin B showed a significant bone

TABLE II. EFFECT OF AMPHOTERICIN B ON THE CALCIUM AND HYDROXYPROLINE REMAINING IN THE CALVARIA AT THE END OF THE EXPERIMENT[a]

Experimental groups	Experiment 1		Experiment 2		Experiment 3	
	Ca content (μg/calv.)	Hydroxyproline (μg/calv.)	Ca content (μg/calv.)	Hydroxyproline (μg/calv.)	Ca content (μg/calv.)	Hydroxyproline (μg/calv.)
Controls	553 ± 30	66 ± 5.0	545 ± 37	62 ± 5.5	608 ± 33	63 ± 6.1
Parathyroid extract (0.1 μ/ml)	390 ± 30**	38 ± 5.0***	320 ± 50*	28 ± 7.8*	459 ± 33**	46 ± 6.1
Amphotericin B (μg/ml)						
50	—	—	225 ± 37*	20 ± 5.5**	240 ± 33***	26 ± 6.1***
20	213 ± 30***	22 ± 5.0***	—	—	—	—
10	160 ± 30***	18 ± 5.0***	265 ± 37**	24 ± 5.5**	257 ± 33***	24 ± 6.1***
5	277 ± 30***	29 ± 5.0***	375 ± 37*	40 ± 5.5*	396 ± 33***	42 ± 6.1*
1	590 ± 30	64 ± 5.0	—	—	568 ± 33	63 ± 6.1
0.5	—	—	620 ± 37	64 ± 7.7	609 ± 33	67 ± 6.1
0.1	583 ± 30	74 ± 5.0	—	—	—	—

[a] Values represent the mean ± SEM.
* $P < 0.05$ as compared to the untreated control.
** $P < 0.01$ as compared to the untreated control.
*** $P < 0.001$ as compared to the untreated control.

TABLE III. EFFECT OF INDOMETHACIN ON AMPHOTERICIN B-INDUCED CALCIUM RELEASE INTO THE CULTURE MEDIUM[a]

Experimental groups	Experiment 1	Experiment 2
Control	2.7 ± 0.71	4.2 ± 0.67
100 ng/ml indomethacin	−1.2 ± 0.71***	0.1 ± 0.67***
100 ng/ml PGE_2	6.3 ± 0.71***	9.9 ± 0.67***
100 ng/ml PGE_2 + 100 ng/ml indomethacin	6.8 ± 0.71***	8.9 ± 0.67***
5 μg/ml amphotericin B	6.2 ± 0.71***	9.5 ± 0.67***
5 μg/ml amphotericin B + 100 ng/ml indomethacin	0.1 ± 0.7** †††	1.3 ± 0.67** †††

[a] Values represent mg/dl (mean ± SEM).
** $P < 0.01$ as compared to the untreated control.
*** $P < 0.001$ as compared to the untreated control.
††† $P < 0.001$ as compared to the corresponding indomethacin-untreated group.

resorption-stimulating effect in bone organ culture. The release of large amounts of calcium into the medium in cultures treated with amphotericin B cannot be explained by a mere leaching out of calcium salts during the culture period. Analysis of residual calcium and hydroxyproline in the calvaria after 7 days of treatment with amphotericin B revealed a significant decrease as compared to the untreated control cultures. These findings, together with the histological observations, clearly indicate that the calcium release into the medium represented bone degradation affecting both bone collagen matrix and bone mineral.

Results obtained in the present study clearly showed that indomethacin strongly inhibited the bone resorption-stimulating effect of all three polyene antibiotics studied. In addition, dexamethasone inhibited amphotericin B-stimulated bone resorption. Since dexamethasone interferes with prostaglandin biosynthesis at the level of phospholipase A_2, which provides prostaglandin precursors (20), and indometha-

TABLE IV. EFFECT OF DEXAMETHASONE ON AMPHOTERICIN B-INDUCED CALCIUM RELEASE INTO THE CULTURE MEDIUM[a]

Experimental groups	Experiment 1	Experiment 2
Control	0.7 ± 0.68	0.6 ± .61
0.1 u/ml PTE	9.1 ± 0.68***	8.8 ± 0.61***
0.1 u/ml PTE + 10^{-8} *M* dexamethasone	3.9 ± 0.68** †††	6.1 ± 0.61*** ††
0.1 u/ml PTE + 10^{-9} *M* dexamethasone	7.2 ± 0.68***	5.5 ± 0.61*** †††
5 μg/ml amphotericin B	11.8 ± 0.68***	9.0 ± 0.61***
5 μg/ml amphotericin B + 10^{-8} *M* dexamethasone	−0.3 ± 0.68 †††	0.4 ± 0.61 †††
5 μg/ml amphotericin B + 10^{-9} *M* dexamethasone	5.0 ± 0.68*** †††	4.8 ± 0.61*** †††

[a] Values represent mg/dl (mean ± SEM).
** $P < 0.01$ as compared to the untreated control.
*** $P < 0.001$ as compared to the untreated control.
†† $P < 0.01$ as compared to the corresponding dexamethasone-untreated group.
††† $P < 0.001$ as compared to the corresponding dexamethasone-untreated group.

TABLE V. EFFECT OF INDOMETHACIN ON NYSTATIN-INDUCED CALCIUM RELEASE INTO THE CULTURE MEDIUM[a]

Experimental Groups	Experiment 1	Experiment 2
Control	−0.8 ± 0.38	1.3 ± 0.51
100 ng/ml indomethacin	−1.6 ± 0.38	—
1 μg/ml PGE_2	—	14.2 ± 0.51***
1 μg/ml PGE_2 + 100 ng/ml indomethacin	—	10.8 ± 0.65***
100 ng/ml PGE_2	5.0 ± 0.42***	—
100 ng/ml PGE_2 + 100 ng/ml indomethacin	5.9 ± 0.42***	—
20 μg/ml nystatin	2.7 ± 0.38**	4.7 ± 0.51***
20 μg/ml nystatin + 100 ng/ml indomethacin	0.9 ± 0.38††	2.3 ± 0.80†
10 μg/ml nystatin	2.7 ± 0.38**	3.5 ± 0.51**
10 μg/ml nystatin + 100 ng/ml indomethacin	−0.1 ± 0.38†††	1.6 ± 0.51†

[a] Values represent mg/dl (mean ± SEM).
** $P < 0.01$ as compared to the untreated control.
*** $P < 0.001$ as compared to the untreated control.
† $P < 0.05$ as compared to the corresponding indomethacin-untreated group.
†† $P < 0.01$ as compared to the corresponding indomethacin-untreated group.
††† $P < 0.001$ as compared to the corresponding indomethacin-untreated group.

cin at the level of cyclooxygenase, which converts prostaglandin precursors to prostaglandin endoperoxides (21), it may be hypothesized that polyene antibiotics stimulate bone resorption in tissue culture by a prostaglandin-mediated mechanism. In this respect, they resemble the membrane active agents, such as the tumor-promoting phorbol diesters and mellitin. These compounds have been shown to stimulate bone resorption in tissue culture accompanied by an increased prostaglandin production. In these experiments, indomethacin inhibited both bone resorption and prostaglandin production (18).

In considering the mechanism of action of polyene antibiotics, it should be noted that amphotericin B binds to cholesterol or some other steroids in cell membranes, thereby modifying the cell membrane and causing an increased permeability (22–25). Apparently, the interaction of amphotericin B and membrane steroids results in the formation of aqueous pores of specific size (about 8 Å in diameter) which facilitate the transport of some ions and other solutes up

TABLE VI. EFFECT OF INDOMETHACIN ON FILIPIN-INDUCED CALCIUM RELEASE INTO THE CULTURE MEDIUM[a]

Experimental groups	Experiment 1	Experiment 2
Control	3.2 ± 0.50	2.2 ± 0.80
100 ng/ml indomethacin	−0.7 ± 0.50***	−1.1 ± 0.80***
100 ng/ml PGE_2	9.2 ± 0.50***	8.2 ± 0.80***
100 ng/ml PGE_2 + 100 ng/ml indomethacin	9.7 ± 0.50***	6.9 ± 0.80***
3.5 μg/ml filipin	8.7 ± 0.50***	6.8 ± 0.80***
3.5 μg/ml filipin + 100 ng/ml indomethacin	1.2 ± 0.50†††	1.6 ± 0.80†††

[a] Values represent mg/dl (mean ± SEM).
*** $P < 0.001$ as compared to the untreated control.
††† $P < 0.001$ as compared to the corresponding indomethacin-untreated group.

to the size of glucose (24, 25). It is possible, therefore, that in the experiments reported here, amphotericin B interacted with bone cell membranes to form pores and increase cellular permeability, thereby triggering the bone resorption process. Along this line, it is of interest that in the same systems nystatin was shown to give rise to membrane pores of somewhat smaller size than those formed by amphotericin B, and that filipin caused a formation of aggregates in the hydrophobic core of the membrane leading to the disruption of the cell membranes (24, 25). Our observation that dexamethasone, a membrane-stabilizing agent (26), inhibits amphotericin B-stimulated bone resorption may be explained on the basis of its blocking cell membrane alterations caused by the polyene antibiotics. Finally, it is generally believed that glucocorticoids inhibit protein synthesis and, therefore, it is conceivable that the inhibition of amphotericin B-stimulated bone resorption by dexamethasone is due to the inhibition of synthesis of proteins necessary for continued resorption. Such a conclusion has been drawn by others about dexamethasone inhibition of PTH-stimulated bone resorption in tissue culture (19).

1. Murray JJ, Reed PW, Fay FS. Contraction of isolated smooth muscle cells by ionophore A23187. Proc Nat Acad Sci USA 72:4459–4463, 1975.
2. Garcia AG, Kirkepar SM, Pratt JC. A calcium ionophore stimulating the secretion of catecholamines from the cat adrenal. J Physiol (London) 244:253–262, 1975.
3. Feinstein MB, Fraser C. Human platelet secretion and aggregation induced by calcium ionophores. Inhibition by PGE_1 and dibutyryl cyclic AMP. J Gen Physiol 66:561–581, 1975.
4. Scheele G, Haymovits A. Potassium- and ionophore A23187-induced discharge of secretory protein in guinea pig pancreatic lobules. Role of extracellular calcium. J Biol Chem 255:4918–4927, 1980.
5. Ahkong QF, Tampion W, Lucy JA. Promotion of cell fusion by divalent cation ionophores. Nature (London) 256:208–209, 1975.
6. Schudt C, Pette D. Influence of the ionophore A23187 on myogenic cell fusion. FEBS Lett 59:36–38, 1975.
7. Dziak R, Stern P. Parathyromimetic effects of the ionophore, A23187, on bone cells and organ cultures. Biochem Biophys Res Commun 65:1343–1349, 1975.
8. Dziak R, Stern PH. Responses of fetal rat bone cells and bone organ cultures to the ionophore, A23187. Calcif Tissue Res 22:137–147, 1976.
9. Eilon G, Raisz LG. Comparison of the effects of stimulators and inhibitors of resorption on the release of lysosomal enzymes and radioactive calcium from fetal bone in organ culture. Endocrinology 103:1969–1975, 1978.
10. Rabadjija L, Goldhaber P. Serum inhibition of ionophore (A23187)-stimulated bone resorption in tissue culture. J Dent Res (special issue A) 60:499, 1981 (Abstr 758).
11. Woessner JF, Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch Biochem Biophys 93:440–447, 1961.
12. Goldhaber P. Bone-resorption factors, cofactors, and giant vacuole osteoclasts in tissue culture. In: Gaillard PJ, Talmage RV, Budy AM, eds. The Parathyroid Glands: Ultrastructure, Secretion, and Function. The University of Chicago, p153, 1966.
13. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature New Biol 231:232–235, 1971.
14. Goldhaber P, Rabadjija L, Beyer WR, Kornhauser A. Bone resorption in tissue culture and its relevance to periodontal disease. J Amer Dent Assoc 87:1027–1033, 1973.
15. Raisz LG, Sandberg AL, Goodson JM, Simmons HA, Mergenhagen SE. Complement-dependent stimulation of prostaglandin synthesis and bone resorption. Science 185:789–791, 1974.
16. Tashjian AH, Jr, Voelkel EF, Levine L, Goldhaber P. Evidence that the bone resorption-stimulating factor produced by mouse fibrosarcoma cells is prostaglandin E_2. A new model for the hypercalcemia of cancer. J Exp Med 136:1329–1343, 1972.
17. Rabadjija L, Goldhaber P. Inhibition by indomethacin of various bone resorption-stimulating factors in tissue culture. J Dent Res (special issue B) 55:B117, 1976 (Abstr 221).
18. Tashjian AH, Jr, Ivey JL, Delclos B, Levine L. Stimulation of prostaglandin production in bone by phorbol diesters and mellitin. Prostaglandins 16:221–232, 1978.
19. Stern PH. Inhibition by steroids of parathyroid hormone-induced Ca^{45} release from embryonic rat bone *in vitro*. J Pharmacol Exp Ther 168:211–217, 1969.
20. Flower RJ, Blackwell GJ. Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. Nature (London) 278:456–459, 1979.
21. Hamberg M, Samuelsson B. Prostaglandin en-

doperoxides. Novel transformations of arachidonic acid in human platelets. Proc Nat Acad Sci USA 71:3400–3404, 1974.
22. Kinsky SC. Antibiotic interaction with model membranes. Annu Rev Pharmacol 10:119–142, 1970.
23. Andreoli TE. The structure and function of amphotericin B-cholesterol pores in lipid bilayer membranes. Ann NY Acad Sci 235:448–468, 1974.
24. De Kruijff B, Gerritsen WJ, Oerlemans A, Demel RA, van Deenen LLM. Polyene antibiotic-sterol interactions in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes. I. Specificity of the membrane permeability changes induced by the polyene antibiotics. Biochim Biophys Acta 339:30–43, 1974.
25. De Kriujff B, Demel RA. Polyene antibiotic–sterol interactions in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes. III. Molecular structure of the polyene antibiotic–cholesterol complexes. Biochim Biophys Acta 339:57–70, 1974.
26. Smith RJ, Sabin C, Gilchrest H, Williams S. Effect of anti-inflammatory drugs on lysosomes and lysososal enzymes from rat liver. Biochem Pharmacol 25:2171–2177, 1976.

Received July 16, 1981. P.S.E.B.M. 1982, Vol. 169.