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STUDIES OF LYSOSOMES—VIII

THE EFFECT OF POLYENE ANTIBIOTICS ON LYSOSOMES*

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Abstract—Polyene antibiotics were added to suspensions rich in lysosomes from rabbit liver, kidney, and leukocytes. Filipin, etruscomycin, and pimaricin and nystatin released acid phosphatase, aryl sulfatase, and β-glucuronidase from lysosomes in rabbit liver and kidney granule suspensions at near-neutral and at acid pH (4·6). They did not release malic dehydrogenase from mitochondria present in the same suspension. Filipin and etruscomycin released β-glucuronidase from rabbit peritoneal leukocyte lysosomes and changed the turbidity of such suspensions. Amphotericin B induced the release of lysosomal enzymes from rabbit kidney granules only at acid pH, but did not induce leakage of malic dehydrogenase. None of the polyenes affected mitochondria, as judged by optical studies of mitochondrial swelling and/or lysis under conditions which led to mitochondrial changes induced by substrates, vitamin A, or thyroxine. These studies suggest that lysosomes from different tissues vary in their susceptibility to a given pharmacologic agent and that they are much more sensitive to polyene antibiotics than mitochondria, which are more responsive to excess of vitamin A. Subcellular organelles can be disrupted by polyenes; this finding supports the hypothesis that polyene antifungal antibiotics act, indiscriminately in a sense, upon the membranes which bound many types of cells and organelles.

POLYENE antibiotics, such as filipin or amphotericin B, appear to kill susceptible organisms by disrupting the boundaries that separate cells from their environment. The site of polyene action upon the cell surface has been further localized to the unit membrane which surrounds cells and organelles.[1] Indeed, strong but indirect evidence suggests that sterols are the unique component of lipid membranes with which these antibiotics react.[2–4] Thus bacteria, the membranes of which lack sterols, are unaffected by polyenes, whereas fungi, which contain ergosterol within their cell membrane, are killed by polyene antibiotics.[1,4] Mycoplasma grown in the presence of cholesterol are susceptible to filipin, but become refractory to the polyene when grown in the absence of a cholesterol source.[5] These studies have been supported by recent evidence that polyene antibiotics lyse mammalian erythrocytes (which contain cholesterol) and that they penetrate monolayers of cholesterol or ergosterol but fail to interact with monolayers of natural or synthetic phospholipids.[6]

However, Ghosh and Chatterjee[7] showed that polyenes interact with phospholipids, and a detailed study of the mechanisms whereby polyenes kill fungi has suggested

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that polyenes may be divided into two groups, depending upon their ring size and overall molecular diameter.[1, 8] Thus the smaller polyenes—filipin, pimaricin, and etruscomycin (mol. wt. 571, 681, 700)—induce irreversible damage to fungal cells, and act more rapidly than larger polyenes such as nystatin or amphotericin (mol. wt. 932, 960).[1, 8] Recent studies support this hypothesis: it was found that smaller polyenes such as filipin induced release of sequestered anions and glucose from phospholipid spherules prepared either in the presence or absence of cholesterol. In direct contrast, the smaller polyenes, amphotericin B and nystatin, preferentially altered the permeability of spherules prepared *with* cholesterol. These large polyenes were less capable of disrupting membranes prepared in the absence of cholesterol.[9]

As an extension of previous studies on the labilization and stabilization of lysosomes it was therefore of interest to examine the effects of polyenes upon these organelles. It was considered likely that differences would be encountered in the action of the two subgroups of polyenes upon lysosomes from different organs, or that lysosomes and mitochondria from the same organ might show differential susceptibility to lysis. These possibilities were suggested by the clinical observations that amphotericin B is not only hemolytic and pyrogenic in man[10] (see Ref. 11 for a discussion of the relationship between exogenous pyrogens and lysosomes) but that this polyene induces renal tubular lesions which have recently been associated with alterations of lysosomal morphology and distribution.[12]

The studies to be detailed below show that polyene antibiotics do indeed disrupt lysosomes but do not affect mitochondria in the same suspension. They further demonstrate that smaller polyenes such as filipin and etruscomycin disrupt lysosomes from liver, kidney, and leukocytes at acid and neutral pH. In contrast, amphotericin B and nystatin were relatively ineffective in releasing lysosomal enzymes from these organelles, except for a significant effect of amphotericin B upon kidney lysosomes at acid pH. These studies therefore suggest that lysosomes in different organs differ in their susceptibility to disruption by pharmacologic agents, and that their surface membranes differ in this property from those of mitochondria.

MATERIALS AND METHODS

Preparation of granule fractions and assay of released enzymes. The preparation of granular fractions from rabbit liver and kidney, sedimenting between 800 *g* (10 min) and 20,000 *g* (20 min) in 0·25 M sucrose, has been described in detail previously.[13] These fractions (5·0 ml) were incubated either in "neutral" unbuffered sucrose and 0·01 M NaCl (pH 6·8 $\pm$ 0·2) or in 0·01 M acetate buffer (pH 4·6) at times indicated below. To chilled tubes containing the granules, 0·05-ml samples of the polyenes, or retinol, in 95% dimethylsulfoxide, were added with constant agitation. After incubation at 37° for periods indicated below, samples were spun at 20,000 *g* for 20 min, and the enzyme activity of the clear supernatants was determined by methods described elsewhere.[13] Aryl sulfatase was measured by the method of Roy.[14] At concentrations of $7{\cdot}5 \times 10^{-4}$ M and 5×10^{-4} M, none of the polyenes inhibited or augmented the activity of the three lysosomal hydrolases, nor of mitochondrial malic dehydrogenase, when these enzymes had been solubilized from liver granules by blending the suspension in a Waring-Blendor and removing the sedimentable debris (20,000 *g*, 20 min).

Mitochondrial swelling or lysis (see Discussion below) was determined by changes in the apparent absorbance of granule fractions from rabbit liver isolated in 0·44 M unbuffered sucrose and resuspended in 0·3 M sucrose containing 0·20 M Tris buffer (pH 7·4). These fractions were studied at 23° after their apparent absorbance had been adjusted to 0·500 at 520 mμ. After completion of the experimental period, samples were spun at 20,000 *g* for 20 min, and the activity of β-glucuronidase, aryl sulfatase, and malic dehydrogenase of the clear supernatant was determined. These procedures and their validation have been described in detail elsewhere.[15]

Rabbit peritoneal leukocyte lysosomes were prepared by a modification of the method of Cohn and Hirsch[16] previously described.[17] Lysis of the granules was followed after they had been suspended in 0·3 M sucrose containing 0·02 M Tris buffer, pH 7·4, and 40 units of U.S.P. heparin (to prevent clumping). Changes in the apparent absorbance of leukocyte lysosomes were measured in a Gilford multiple-sample absorbance spectrophotometer. Readings were taken of blanks, controls containing DMSO, Triton X-100-treated, and polyene-treated granules, at 1-min intervals. After 5 min, the samples were spun at 20,000 *g* for 20 min and the β-glucuronidase activity of the clear supernatant was determined.

Reagents. Amphotericin B and nystatin were obtained from the Squibb Institute for Medical Research, New Brunswick, N.J.; etruscomycin from Farmitalia, Milan, Italy; filipin from Upjohn Co., Kalamazoo, Mich.; and pimaricin from Lederle Laboratories, Pearl River, N.Y. These were freshly prepared in DMSO daily; otherwise they are unstable in solution. DMSO and thyroxine, and retinol (vitamin A) were obtained from Sigma Biochemicals, St. Louis, Mo., and Triton X-100 from Rohm & Haas, Philadelphia, Pa.

RESULTS

Release of enzymes from rabbit liver granules

The effect of increasing concentrations of polyene antibiotics upon release of β-glucuronidase, aryl sulfatase, and malic dehydrogenase from liver granules incubated for 60 min in unbuffered sucrose may be seen in Figs. 1 and 2. Filipin and etruscomycin were most effective in releasing aryl sulfatase and β-glucuronidase; filipin manifested activity at 5×10^{-5} M. Pimaricin and nystatin were relatively less active under these conditions; amphotericin released little soluble enzyme activity. Malic dehydrogenase, the mitochondrial "marker", was not released by filipin at concentrations that induced maximal solubilization of aryl sulfatase, and β-glucuronidase from lysosomes.

To determine whether this relative insusceptibility of mitochondria to polyenes was evident under other conditions, granules were incubated for various times at 37°, at pH 4·6. These conditions favor breakdown of mitochondria and lysosomes.[11, 15] Figure 3 shows results of experiments when filipin and amphotericin were compared with the polyene vitamin, retinol (vitamin A), an agent that disrupts lysosomes and mitochondria.[15] Whereas retinol (5×10^{-4} M) released malic dehydrogenase from mitochondria to a greater extent than acid phosphatase from lysosomes, filipin (5×10^{-5} M) released only acid phosphatase. It should be noted that the concentrations of filipin required for lysosomal disruption are among the lowest described for *any* labilizer of lysosomes.[11] Amphotericin B (5×10^{-4} M) was ineffective in solubilizing either acid phosphatase or malic dehydrogenase.

A summary of experiments in which polyenes were added to liver granules is seen in Table 1. Because of the sharp increment in lysosome-disruptive capacity of several agents between 10^{-4} and 10^{-3} M, the antibiotics were added at 5×10^{-4} M. At both pH 6·8 and 4·6 the polyenes appeared to solubilize lysosomal hydrolases

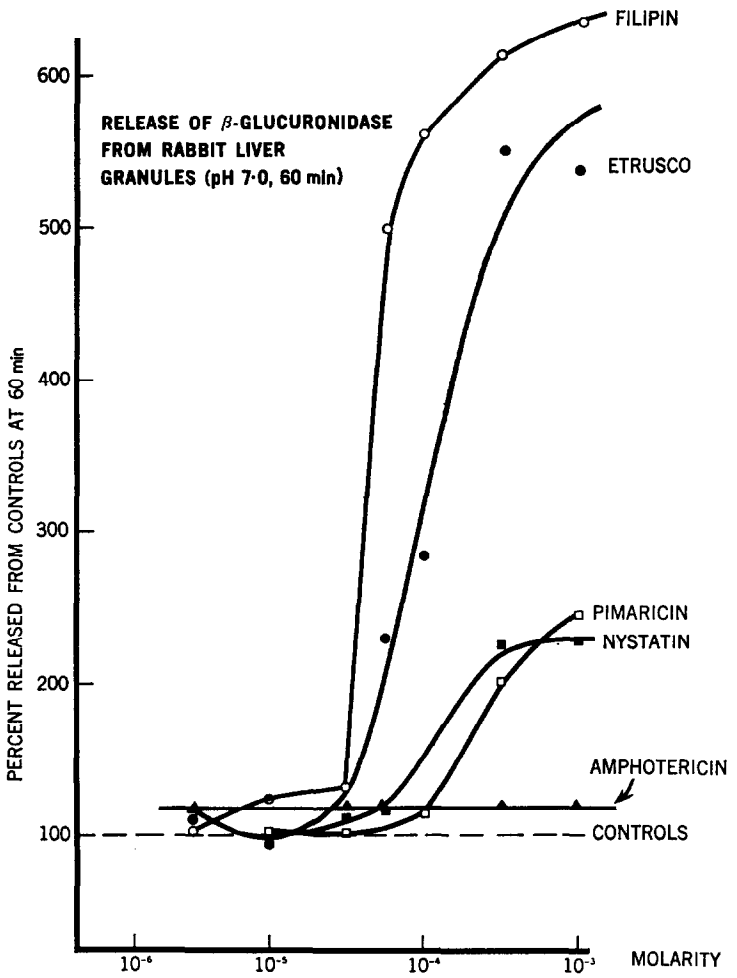

FIG. 1. Effect of increasing concentrations of polyenes upon release of β-glucuronidase from rabbit liver granules. Polyenes in DMSO (1% v/v) or DMSO were added for 60 min to liver granules in 0·25 M sucrose. Granules were spun at 20,000 *g* for 20 min, and enzyme activity released into the supernatant was measured.

(acid phosphatase, β-glucuronidase, and aryl sulfatase) in the following order: filipin > etruscomycin > pimaricin > nystatin > amphotericin B. The last two were least effective, and belong to the larger molecular weight group of polyenes. None of the antibiotics released significant amounts of malic dehydrogenase from mitochondria.

Release of enzymes from rabbit kidney granules

Because renal lesions are a major manifestation of amphotericin B toxicity in humans,[10] the effect of polyene antibiotics was studied on rabbit kidney granules. Data shown in Fig. 4 show that filipin, etruscomycin, and amphotericin, in that

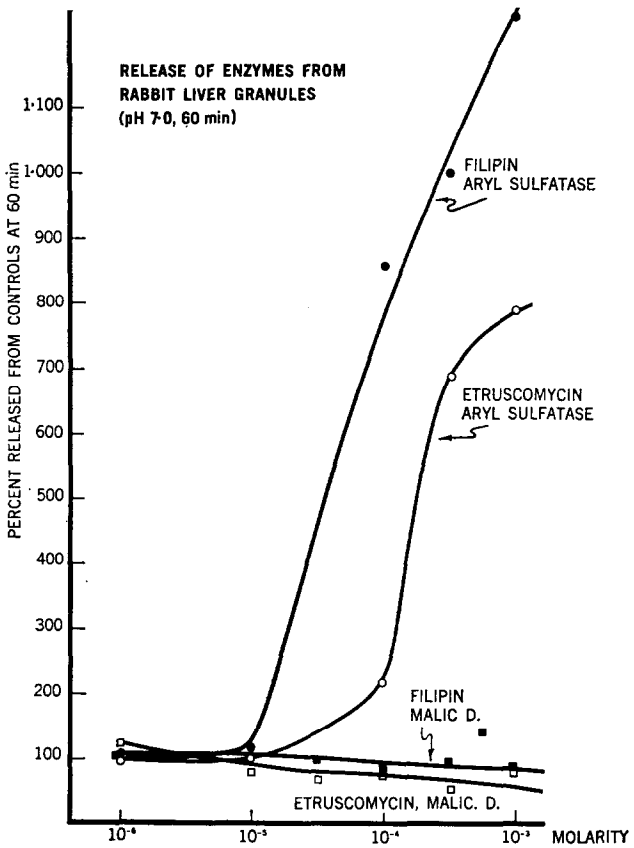

FIG. 2. Effect of increasing concentrations of filipin and etruscomycin upon release of aryl sulfatase from lysosomes and malic dehydrogenase from mitochondria in rabbit liver granules. Conditions as in Fig. 1.

order, released significant amounts of β-glucuronidase and acid phosphatase activity into the supernatants of granular fractions incubated for 20 min at pH 4·6. Filipin and etruscomycin were effective above concentrations of 5×10^{-5} M, amphotericin above 10^{-4} M. Even at concentrations of polyenes which provoked maximal release of lysosomal enzymes, no significant solubilization of malic dehydrogenase was observed.

In Table 2 is a summary of experiments with rabbit kidney granules at pH 6·8 and 4·6. At pH 6·8, the relative order of polyene activity was filipin > etruscomycin > pimaricin > amphotericin > nystatin. Pimaricin was relatively more effective in

promoting release of lysosomal hydrolases from kidney granules than from those obtained from liver, its activity was noted both at near-neutrality and at more acid pH. In direct contrast, amphotericin B was capable of inducing significant release of lysosomal enzymes from kidney granules only at the lower pH.

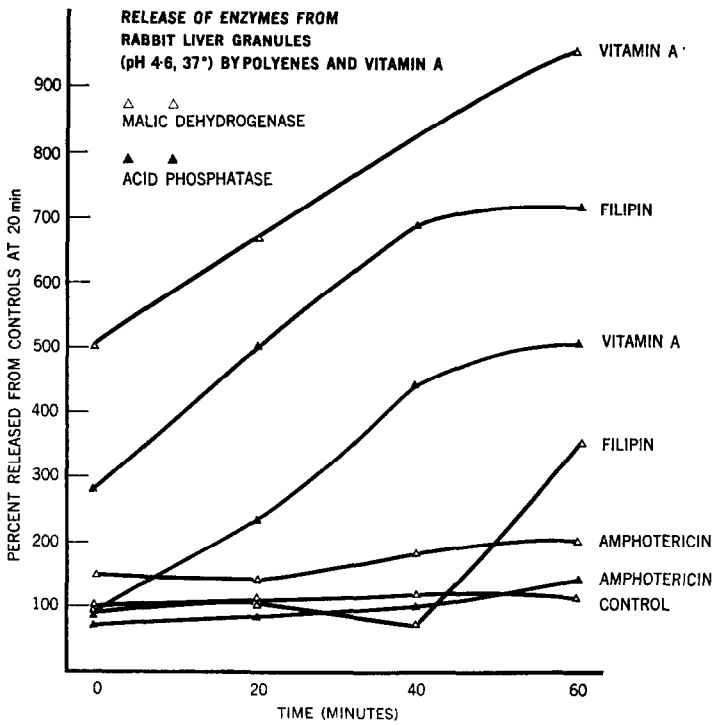

FIG. 3. Effect of polyene antibiotics and retinol (vitamin A) on release of acid phosphatase (▲—▲) from lysosomes and malic dehydrogenase (△—△) from mitochondria in rabbit liver granules. Filipin (5×10^{-5} M), amphotericin (5×10^{-4} M), vitamin A (5×10^{-4} M) added at time 0, ph 4·6. Granule fractions were removed at times indicated and spun at 20,000 *g* for 20 min, and enzyme activity released into supernatant was measured.

Release of enzymes from rabbit leukocyte lysosomes

It is possible to study the response to lytic agents of a relatively pure (85–95 per cent) suspension of leukocyte lysosomes to lytic agents.[17] Leukocyte lysosomes were suspended in Tris buffer (containing heparin to disperse the granules), and the polyenes were added in DMSO (Fig. 5). Under these conditions (at 23°), only filipin etruscomycin, and pimaricin induced changes in the apparent absorbance of granule suspensions. Physical disruption was also observed by phase-contrast microscopy. Filipin and etruscomycin released β-glucuronidase from the granules to an extent comparable to that released by Triton X-100; however, changes in turbidity of the suspension induced by pimaricin were not associated with enzyme release. Under these conditions, and at concentrations of 5×10^{-5} M, amphotericin and nystatin did not release enzymes from leukocyte granules, nor did they induce changes in

TABLE 1. RELEASE OF ENZYMES FROM RABBIT LIVER GRANULES BY POLYENE ANTIBIOTICS (5×10^{-4} M)

Agent	No. of expts.	pH 6·8* Acid phosphatase	pH 6·8* β-Glucuronidase	pH 6·8* Aryl sulfatase	pH 6·8* Malic dehydrogenase	pH 4·6† Acid phosphatase	pH 4·6† β-Glucuronidase	pH 4·6† Aryl sulfatase	pH 4·6† Malic dehydrogenase
Filipin	8	629‡ ± 31	676‡ ± 43	1,003‡ ± 89	098 ± 10	588‡ ± 43	428‡ ± 39	427‡ ± 58	103 ± 09
Etruscomycin	8	375‡ ± 23	500‡ ± 54	510‡ ± 69	112 ± 14	210‡ ± 24	271‡ ± 38	224‡ ± 40	089 ± 15
Pimaricin	6	168‡ ± 15	184‡ ± 18	189‡ ± 22	098 ± 12	162‡ ± 17	152§ ± 21	131§ ± 12	087 ± 11
Amphotericin B	8	100 ± 21	111 ± 10	116 ± 21	092 ± 13	118 ± 14	115 ± 20	096 ± 14	088 ± 10
Nystatin	6	126 ± 20	177§ ± 14	152§ ± 11	088 ± 24	141§ ± 11	183§ ± 13	150§ ± 17	102 ± 10
Controls¶	24	100 (9·3 ± 1·8)	100 (10·4 ± 2·1)	100 (8·9 ± 1·5)	100 (24·5 ± 8·1)	100 (15·5 ± 6·2)	100 (14·3 ± 4·2)	100 (12·9 ± 3·8)	100 (15·5 ± 3·1)

* Granules incubated 50 min at 37°, results expressed as per cent of enzyme activity released from control suspensions ± S.E.M.

† Granules incubated 20 min at 37°, results expressed as above.

‡ < P 0·01 vs. matched controls.

§ < P 0·05 vs. matched controls.

¶ Figures in parentheses represent activities released from control granules at either pH expressed as per cent of total enzyme activity measured after disruption of all granules by Triton X-100 (0·1 v/v).

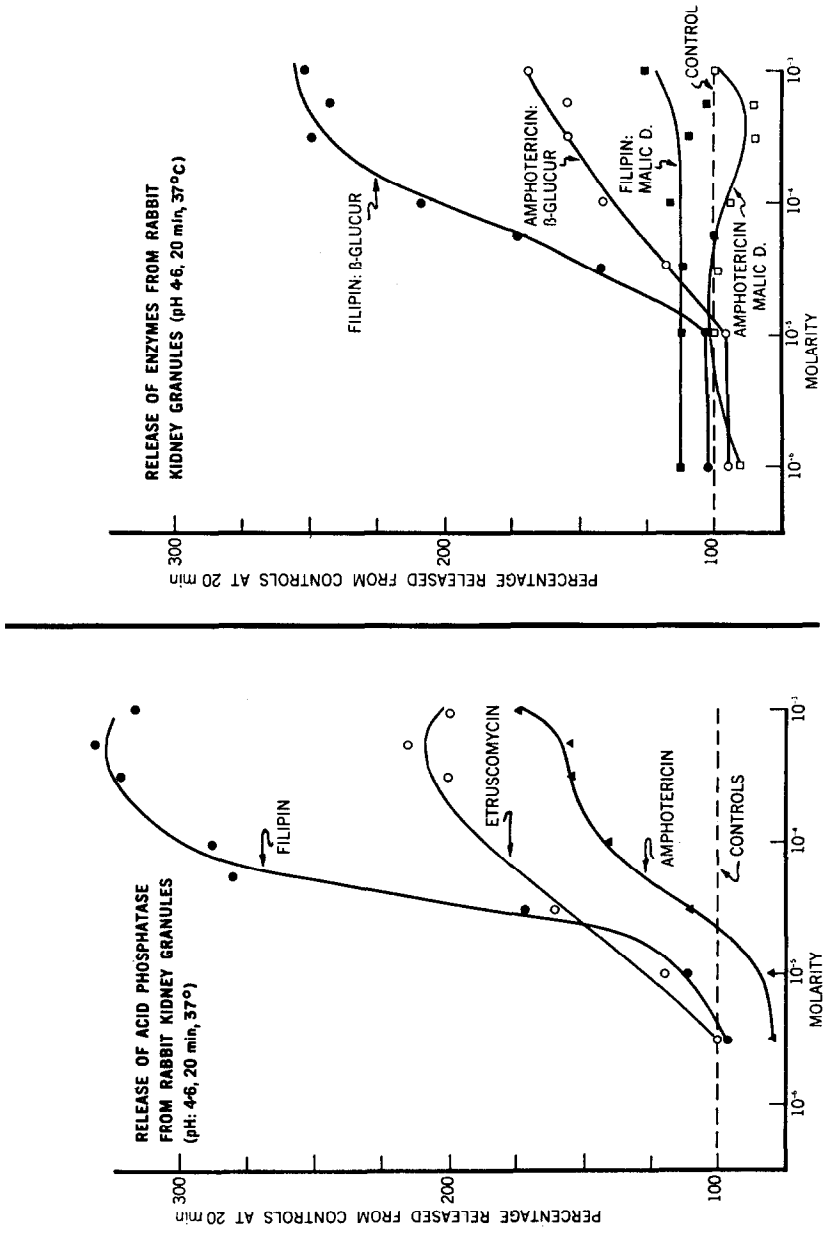

FIG. 4. Effect of polyene antibiotics on release of acid phosphatase and β-glucuronidase (β-glucur.) from lysosomes, and malic dehydrogenase (malic d.) from mitochondria in rabbit kidney granules. Conditions as in Fig. 3, except 20-min incubation time. All polyenes present at 5×10^{-4} M.

TABLE 2. RELEASE OF ENZYMES FROM RABBIT KIDNEY GRANULES BY POLYENE ANTIBIOTICS (5×10^{-4} M)

		pH 6·8*				pH 4·6†			
Agent	No. of expts.	Acid phosphatase	β-Glucuro-nidase	Aryl sulfatase	Malic dehydro-genase	Acid phosphatase	β-Glucuro-nidase	Aryl sulfatase	Malic dehydro-genase
Filipin	8	580‡ ± 28	295‡ ± 18	533‡ ± 39	098 ± 08	382‡ ± 21	253‡ ± 14	389‡ ± 43	101 ± 12
Etruscomycin	6	560‡ ± 40	313‡ ± 21	543‡ ± 43	087 ± 12	200‡ ± 10	187‡ ± 18	205‡ ± 31	104 ± 15
Pimaricin	6	270‡ ± 32	287‡ ± 11	307‡ ± 36	086 ± 06	272‡ ± 31	156‡ ± 13	188‡ ± 21	079 ± 12
Amphotericin B	8	116 ± 04	114 ± 09	110 ± 08	102 ± 15	177‡ ± 08	154‡ ± 06	182‡ ± 05	089 ± 07
Nystatin	6	140§ ± 11	146§ ± 15	156§ ± 08	082 ± 13	100 ± 06	103 ± 08	107 ± 09	103 ± 12
Controls¶	36	100 (14·3 ± 2·3)	100 (12·2 ± 3·5)	100 (14·5 ± 3·7)	100 (17·5 ± 4·8)	100 (18·9 ± 3·4)	100 (19·8 ± 2·1)	100 (16·3 ± 2·5)	100 (14·3 ± 2·9)

* Granules incubated 50 min at 37°, results expressed as per cent of enzyme activity released from control suspensions ± S.E.M.

† Granules incubated 20 min at 37°, results expressed as above.

‡ < P 0·01 vs. matched controls.

§ < P 0·05 vs. matched controls.

¶ Figures in parentheses represent activities released from control granules at either pH expressed as per cent of total enzyme activity measured after distruption of all granules by Triton X-100 (0·1 v/v).

turbidity. Higher concentrations of the polyenes caused opalescence of the medium, because of their relative insolubility at room temperature. Acidification of the medium to below 5·5 induced clumping of the granules. Therefore granules were not studied optically at low pH or with higher concentrations of polyenes.

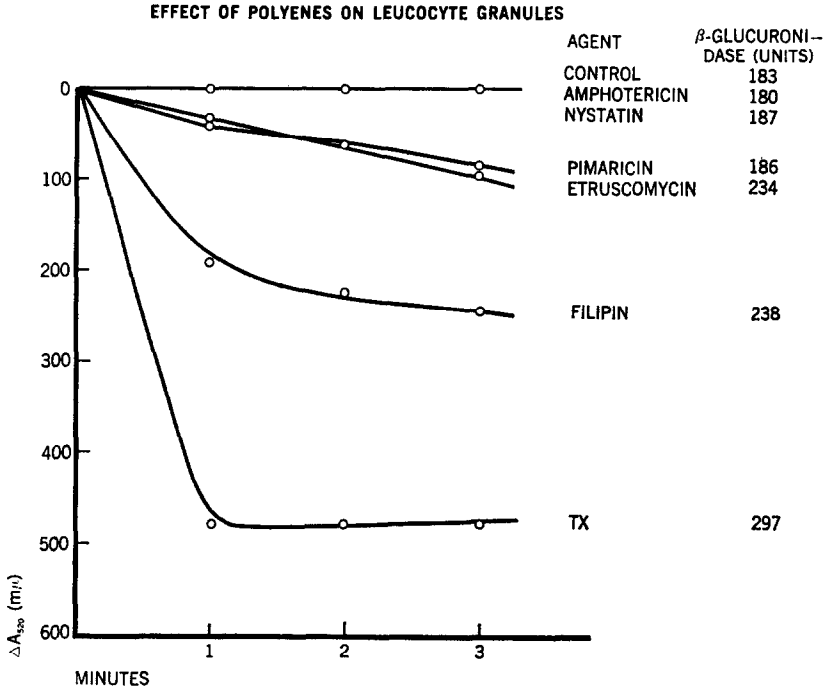

FIG. 5. Effect of polyene antibiotics on turbidity ($\Delta A_{520m\mu}$) and release of enzyme from rabbit leukocyte lysosomes. Polyene antibiotics (5×10^{-5} M) in DMSO (1% v/v) or DMSO alone added to leukocyte lysosomes suspended in 0·3 M Tris (0·02 M) buffer, pH 7·4, to an apparent absorbance of 0·500. Changes in absorbance recorded (23°) at 1-min intervals; after 5 min, granules spun at 20,000 *g* for 20 min at 4° and β-glucuronidase activity (expressed as raw absorbancy of chromogen) determined. TX = Triton X-100 (0·1 v/v).

Changes in the turbidity of, and release of enzyme from a granular fraction of rabbit liver

Optical studies of a granular fraction from rabbit liver may be performed in Tris-buffered sucrose and largely represent physical changes undergone by mitochondria which represent the vast majority of organelles in such suspensions. Changes in the apparent absorbance of this fraction at 520 mμ either represent energy-dependent uptake of water as induced by thyroxine or substrate (e.g. succinate), or represent true lysis of mitochondrial membranes, as induced by retinol.[15] Lysis is accompanied by release of malic dehydrogenase from mitochondria. In Table 3 are shown experiments in which these parameters of mitochondrial integrity are compared with polyene-induced release of β-glucuronidase and aryl sulfatase from lysosomes. These constitute the minority of organelles in granule fractions from liver. At 23°, filipin and etruscomycin induced release of lysosomal hydrolases. These small polyenes did

not provoke physical changes in mitochondria, as judged by their failure to decrease the apparent absorbance of mitochondrial suspensions and their inability to solubilize malic dehydrogenase. No other polyenes released lysosomal hydrolases at 23°, nor did they perturb mitochondria. In direct contrast, and as previously shown, thyroxine and substrate induced mitochondrial swelling unaccompanied by release of malic

TABLE 3. CHANGES IN TURBIDITY OF, AND RELEASE OF ENZYMES FROM, A MITOCHONDRIAL/LYSOSOMAL FRACTION OF RABBIT LIVER

Agent	Concn.	A_{520}*	Malic dehydrogenase†	β-Glucuronidase†	Aryl sulfatase†
Control		0·046	0·053	0·248	0·100
Filipin	5×10^{-5}	0·053	0·050	0·535	0·175
	5×10^{-4}	turbid	0·011	0·940	0·347
Etruscomycin	5×10^{-5}	0·065	0·056	0·464	0·160
	5×10^{-4}	turbid		1·195	0·271
Amphotericin	5×10^{-5}	0·061	0·053	0·237	0·090
	5×10^{-4}	turbid	0·011	0·162	0·086
Pimaricin	5×10^{-5}	0·047	0·058	0·221	0·093
	5×10^{-4}	turbid	0·011	0·187	0·114
Nystatin	5×10^{-5}	0·065	0·041	0·217	0·104
	5×10^{-4}	turbid	0·025	0·193	0·114
Vit. A	5×10^{-5}	0·192	0·193	0·231	0·124
	3×10^{-4}	turbid		0·900	
Thyroxin	5×10^{-5}	0·184	0·043	0·176	
Succinate	3×10^{-4}	0·207	0·058	0·276	

* Decrease of apparent absorbance at 520 mμ after 30 min at 23°; suspension in 0·3 M sucrose, 0·02 M Tris buffer, ph 7·4.

† Enzyme units (see text).

dehydrogenase from mitochondria. In direct contrast, retinol induced lysis of mitochondria (i.e. changes in apparent absorbance accompanied by solubilization of malic dehydrogenase) at 5×10^{-5} M, while releasing β-glucuronidase at 5×10^{-4} M. These observations also indicate that lysosomes are more susceptible to disruption by polyenes than are mitochondria, which appear to be more sensitive to vitamin A.[15, 17]

DISCUSSION

The data presented above clearly show that polyene antibiotics release hydrolytic enzymes from lysosomes. These agents have previously been shown capable of disrupting the membranes of fungi, appropriately cultured mycoplasma, protozoa, amphibian epithelium, and erythrocytes.[1–5, 18, 19] Thus lysosomes are only the latest in a group of membrane-bounded structures that can be affected by polyenes. Polyene antibiotics appear to interact, indiscriminately in a sense, with membranes common to microorganisms and to host, thereby standing in contrast to more selective antimicrobials such as penicillin.

It is noteworthy that mitochondria were unaffected by polyenes under conditions that favored almost complete disruption of lysosomes. Lardy *et al.* found that polyene antibiotics failed to decrease the P/O ratio of mitochondria,[20] while Kinsky *et al.*[21] demonstrated that earlier reports of actions upon the respiratory enzymes of

mitochondria by polyene antibiotics were complicated by contamination of the antibiotic preparations with antimycin A. However Kinsky *et al.* presented evidence that polyenes are bound to mitochondria, despite the inability of these agents to affect mitochondrial function. Thus the interaction of polyenes *with*, and their effects upon the permeability *of*, biological structures may not be synonymous. Ample evidence has documented interactions of polyenes with purified lipids common to cells and organelles.[1-7] Therefore the differential susceptibility to polyene antibiotics of lysosomes might suggest that the surface lipids of mitochondria lack the optimum phospholipid/cholesterol ratio necessary for the action of polyenes (suggested by Kinsky *et al.*[21] Alternatively, proteins and polysaccharides present on the surfaces of mitochondria (but not of lysosomes in the same suspension) may bind the antibiotics and prevent their access to lipid layers. Another possibility is that mitochondrial swelling and release of malic dehydrogenase is regulated by the inner membranes of mitochondria.[22] Uptake of polyenes by the outer membranes, which play a passive role in mitochondrial swelling and lysis, may prevent interaction of the antibiotics with the inner, or critical, membranes.

Our data show that lower molecular weight polyenes (filipin, etruscomycin, and pimaricin) induced the most drastic changes in lysosomal integrity, whereas the larger polyenes (amphotericin B and nystatin) were relatively less disruptive. Such observations agree with the suggestion of Lampen and Arnow[8] that these two groups affect membranes in dissimilar fashion. This hypothesis has received further support from recent studies of model lipid structures.[9] Studies of enzyme release from lysosomes are comparatively insensitive. They measure irreversible disruption of the integrity of lysosomal membranes with leakage of enzyme protein. In the case of other lysosome-disruptive procedures, such gross changes are preceded by (?reversible) increases in the permeability of relatively intact membranes to smaller molecular weight materials such as substrates.[23] These more subtle changes in permeability of membranes can be studied in model systems of artificial lipids and these have indeed shown that amphotericin B and nystatin promote greater diffusion of anions and glucose from spherules of phospholipid prepared with cholesterol. Smaller polyenes did not require the presence of cholesterol for their membrane-disruptive activity to be demonstrated in artificial systems.[9]

It would appear that lysosomes from different tissues display different susceptibilities to disruption by the same polyene. Lysosomes from liver were disrupted by filipin, etruscomycin, and pimaricin, but *not* by amphotericin B. In contrast, amphotericin B released significant amounts of lysosomal hydrolases from kidney granules, especially at acid pH. Kidney lysosomes also appeared more susceptible to the action of pimaricin; this was evident at near-neutrality and at acid pH. It is conceivable that the augmented response of kidney lysosomes to amphotericin B at acid pH may play a role in the renal lesions produced by this antibiotic. Tubular cells from kidneys of animals treated with amphotericin B are filled with remains of altered lysosomes: residual bodies, autophagic vacuoles, etc.[12] Furthermore, the repeated administration of amphotericin B to humans is regularly attended by brisk pyrogenic responses,[10] resembling in onset and course the recurrent fever provoked by another pyrogen, etiocholanolone, a steroid that disrupts lysosomes *in vitro*.[11] Such indirect evidence should stimulate further efforts to relate the pyrogenicity and renal toxicity of amphotericin B to effects of this antibiotic upon lysosomes.

REFERENCES

1. J. O. Lampen, *Symp. Soc. gen. Microbiol.* **16**, 111 (1966).
2. D. Gottlieb, H. E. Carter, L. Wu and J. H. Sloneker, *Phytopathology* **50** 594 (1960).
3. S. C. Kinsky, *Antimicrob. Agents Annual*, 1963, p. 387.
4. J. O. Lampen and P. M. Arnow, *Proc. Soc. exp. Biol. Med.* **101**, 792 (1959).
5. M. M. Weber and S. C. Kinsky, *J. Bact.* **89**, 306 (1965).
6. R. A. Demel, L. L. M. Van Deenen and S. C. Kinsky, *J. biol. Chem.* **240**, 2749 (1965).
7. B. K. Ghosh and A. N. Chatterjee, *Ann. Biochem. exp. Med.* **23**, 173 (1963).
8. J. O. Lampen and P. M. Arnow, *Bull. Res. Coun. Israel* **11A**, 286 (1963).
9. G. Wiessmann and G. Sessa, *J. biol. Chem.* **242**, 616 (1967).
10. J. P. Utz, *Am. J. Med.* **39**, 826 (1965).
11. G. Wiessmann, *New Engl. J. Med.* **273**, 1084, 1143 (1965).
12. D. G. Scarpelli, J. E. Smith and L. Fulmer, *Am. J. Path.* **44**, 32a (1964).
13. G. Weissmann, H. Keiser and A. W. Bernheimer, *J. exp. Med.* **118**, 205 (1963).
14. A. B. Roy, *Biochem. J.* **68**, 519 (1959).
15. H. Keiser, G. Weissmann and A. W. Bernheimer, *J. cell. Biol.* **22**, 101 (1964).
16. Z. A. Cohn and J. G. Hirsch, *J. exp. Med.* **112**, 983 (1960).
17. G. Weissmann, B. Becher and L. Thomas, *J. cell. Biol.* **22**, 115 (1964).
18. N. S. Lichtenstein and A. Leaf, *J. clin. Invest.* **44**, 1328 (1965).
19. S. C. Kinsky, *Archs. Biochem. Biophys.* **102**, 180 (1963).
20. H. A. Lardy, D. Johnson and W. C. McMurray, *Archs Biochem. Biophys* **78**, 587 (1958).
21. S. C. Kinsky, G. R. Gronau and M. M. Weber, *Molec. Pharmac.* **1**, 190 (1965).
22. A. L. Lehninger, *Physiol. Rev.* **42**, 467 (1962).
23. S. Shibko, J. Pangborn and A. L. Tappel, *J. cell Biol.* **25**, 479 (1965).