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EFFECTS OF AMPHOTERICIN B ON THE PERMEABILITY OF THE SMALL AND LARGE INTESTINES OF *TESTUDO HERMANNI*

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SUMMARY

1. The effects of Amphotericin B on the permeability *in vitro* of preparations of the small and large intestines of *Testudo hermanni* have been studied.

2. The permeability of the small intestine to thiourea was slightly affected by the presence of polyene in the mucosal fluid, whereas the addition of the same substance greatly increased the permeability of the large intestine to thiourea.

3. Thiourea permeability across the two epithelia was not modified when Amphotericin B was present in the serosal fluid.

4. Most probably these differences depended on different cell membrane structure or composition.

5. Phenylacetic acid permeability was not affected by the addition of Amphotericin B to the mucosal fluid of either epithelium. Probably polyene causes molecular rearrangements without deep disorganization of these membranes.

INTRODUCTION

Polyene antibiotics are believed to cause lysis in fungi by a selective interaction with membrane sterols[1]. Recently the effect of these substances on metazoan cells such as erythrocytes[2] or toad bladder epithelium[3] has been studied by a number of authors. The action of Amphotericin B on red cells is as strong as in fungi, whereas the same polyene seems to affect toad bladder permeability more slightly, and to mimic some effects of a physiological hormone (anti-diuretic hormone). Furthermore, the influence of polyene antibiotics on artificial systems, such as lipid monolayer or bilayer membranes, has also been studied[4,5]. These works have confirmed that the basis of the action of polyenes on permeability is a selective binding with sterols, resulting in a structural reorientation of sterol molecules. According to these findings, polyene antibiotics seem to be indirect but interesting tools for the investigation of the role of sterols in the structure and function of biological membranes.

In the present work a comparison is made between the effects of Amphotericin B on the permeability of two epithelia having the same embryological origin, but different functions, *i.e.* small and large intestine.

METHODS

The epithelial layer of the small and large intestines of *Testudo hermanni* was isolated according to BAILLIEN AND SCHOFFENIELS[6] and put between 2 lucite chambers of the same volume (6 ml); the apparent exposed surface of the epithelia was 1.33 cm^2. The perfusion fluids were gassed with O_2 and kept at constant temperature ($27 \pm 1°$). Their composition was the same as described in a previous work[7]. The experiments were performed during the summer.

In the first set of experiments we studied permeability to a non-ionic, water-soluble substance, thiourea, which is not metabolized by the intestine. [^{14}C]Thiourea efflux (serosa–mucosa) was used as a measure for transepithelial permeability.

The perfusion fluids were thiourea–Ringer[7] on both sides, the serosal one containing [^{14}C]thiourea with a final activity of about 1 μC/ml. After a 2-h equilibration period the radioactivity appearing at the mucosal side during 6 successive 0.5-h periods was measured by emptying the mucosal chamber of the perfusion fluid it contained. The first two periods were taken as a control. Amphotericin B (Fungizone Squibb) at a concentration of 10 μg/ml was added to the mucosal or serosal fluid and the experiment was carried out for the four successive periods.

A second set of experiments was run with the same technique, but with [1-^{14}C]-creatinine as a test substance.

In a third set of experiments we studied transepithelial permeability of phenylacetic acid, a substance mainly soluble in lipid solvents. The perfusion fluids were Turtle–Ringer[7] *plus* 0.5 mmole/l of phenylacetic acid. [1-^{14}C]Phenylacetic acid was added to the serosal fluid with a final activity of about 1 μC/ml and the experiment was carried out as before.

In a fourth set of experiments we employed the washing-out technique according to LIPPE *et al.*[7]. The tissue was put between 2 lucite chambers under the previously reported conditions and perfused for 2 h with thiourea–Ringer; the serosal fluid contained [^{14}C]thiourea with an activity of about 20 μC/ml. At the end of this preloading period the perfusion fluids were recovered and the chambers thoroughly washed with non-radioactive Ringer. 6 ml of the same non-radioactive fluid were then introduced into both chambers and replaced every 10 or 20 min over a 2-h period. After 3 or 4 control periods, thiourea–Ringer with added Amphotericin B (10 μg/ml) was introduced into the mucosal or serosal chamber until the experiment was over.

The radioactivity readings were performed with a Tri-Carb liquid scintillation spectrometer (3000 series).

RESULTS

Table I shows the effect of Amphotericin B on thiourea permeability across the small intestine. The presence of this substance at the mucosal side slightly increased permeability, whereas no effects were present when it was added to the serosal fluid. The mucosal action of polyene was negligible when compared to that obtained in the bladder (LICHTENSTEIN AND LEAF[3]) and large intestine (Table II). Even by increasing Amphotericin B concentration in the mucosal fluid, the resulting increase in thiourea permeability was lower than that obtained in the bladder[3] or large intestine.

TABLE I

THIOUREA EFFLUX ACROSS THE SMALL INTESTINE IN THE PRESENCE OF AMPHOTERICIN B

Φ_A = thiourea efflux in the presence of Amphotericin B. Φ_C = thiourea efflux in control periods. The figures in parentheses represent the number of experiments.

System		*Period*	Φ_0* ($\mu mole \cdot cm^{-2} \cdot h^{-1}$)	$\frac{\Phi_A - \Phi_C}{\Phi_C} \times 100$**
Control	(5)	1st	0.78	
		2nd	0.74	
Amphotericin B,		1st	0.90	23.0 ± 7.0
10 μg/ml in the		2nd	1.00	36.6 ± 7.8
mucosal fluid	(5)	3rd	0.96	31.4 ± 9.3
		4th	0.98	32.3 ± 6.6
Control	(5)	1st	0.64	
		2nd	0.62	
Amphotericin B,		1st	0.85	42.7 ± 12.5
20 μg/ml in the		2nd	0.98	64.1 ± 12.5
mucosal fluid	(5)	3rd	0.96	60.6 ± 16.1
		4th	0.92	55.2 ± 16.6
Control	(4)	1st	0.90	
		2nd	0.90	
Amphotericin B,		1st	0.88	−2.0 ± 1.8
10 μg/ml in the		2nd	0.86	−4.2 ± 1.9
serosal fluid	(4)	3rd	0.92	+2.7 ± 3.4
		4th	0.85	−5.0 ± 3.0

* Mean value.
** Mean value ± S.E.

TABLE II

THIOUREA EFFLUX ACROSS THE LARGE INTESTINE IN THE PRESENCE OF AMPHOTERICIN B

Φ_A = thiourea efflux in the presence of Amphotericin B. Φ_C = thiourea efflux in control periods. The figures in parentheses represent the number of experiments.

System		*Period*	Φ_0* ($\mu moles \cdot cm^{-2} \cdot h^{-1}$)	$\frac{\Phi_A - \Phi_C}{\Phi_C} \times 100$**
Control	(5)	1st	0.28	
		2nd	0.26	
Amphotericin B,		1st	0.94	284.5 ± 56.1
10 μg/ml in the		2nd	1.43	485.3 ± 76.0
mucosal fluid	(5)	3rd	1.53	519.5 ± 87.0
		4th	1.50	515.4 ± 115.5
Control	(4)	1st	0.23	
		2nd	0.22	
Amphotericin B,		1st	0.20	−6.1 ± 2.3
10 μg/ml in the		2nd	0.22	+1.8 ± 11.4
serosal fluid	(4)	3rd	0.20	−6.8 ± 9.5
		4th	0.20	−5.0 ± 8.9

* Mean value.
** Mean value ± S.E.

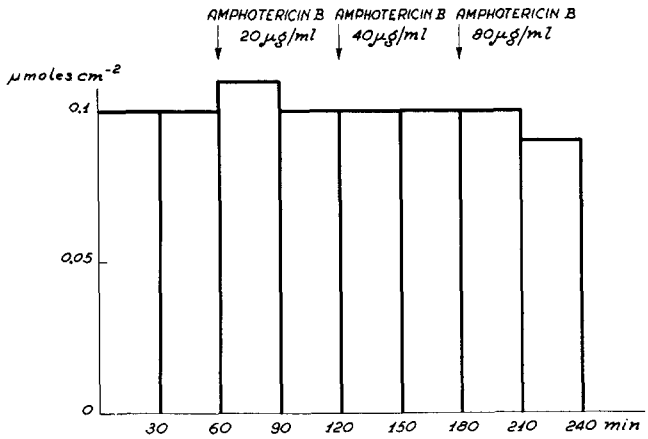

Fig. 1. Thiourea efflux across the large intestine in the presence of Amphotericin B. After two control periods polyene was added to the serosal fluid at increasing concentrations.

The addition of polyene to the large intestine mucosal fluid caused a dramatic increase in thiourea permeability (Table II) similar to that obtained in toad bladder[3]. But in this tissue, as in the case of the small intestine, polyene had no effect when added to the serosal side and even a large increase in Amphotericin B concentration did not affect thiourea permeability (Fig. 1).

In order to evaluate separately the effects of Amphotericin B on thiourea permeability of mucosal or serosal cell membranes, we studied the action of polyene on the washing-out fluxes as illustrated in Fig. 2. Our results confirm the hypothesis that the mucosal membranes of the small and large intestines are differently affected by polyene and that permeability of the serosal cell membranes of both tissues are not modified by polyene addition.

Table III shows the action of Amphotericin B on the permeability of creatinine

TABLE III

CREATININE EFFLUX ACROSS THE LARGE INTESTINE IN THE PRESENCE OF AMPHOTERICIN B IN THE MUCOSAL FLUID

Φ_A = creatinine efflux in the presence of Amphotericin B. Φ_C = creatinine efflux in control periods. The figures in parentheses represent the number of experiments.

System		*Period*	Φ_0* ($\mu mole \cdot cm^{-2} \cdot h^{-1}$)	$\frac{\Phi_A - \Phi_C}{\Phi_C} \times 100$**
Control	(5)	1st	0.052	
		2nd	0.050	
Amphotericin B,		1st	0.063	
10 μg/ml	(5)	2nd	0.092	29.6 ± 6.1
		3rd	0.098	84.9 ± 8.7
		4th	0.108	125.3 ± 21.0
				118.4 ± 19.8

* Mean value.
** Mean value ± S.E.

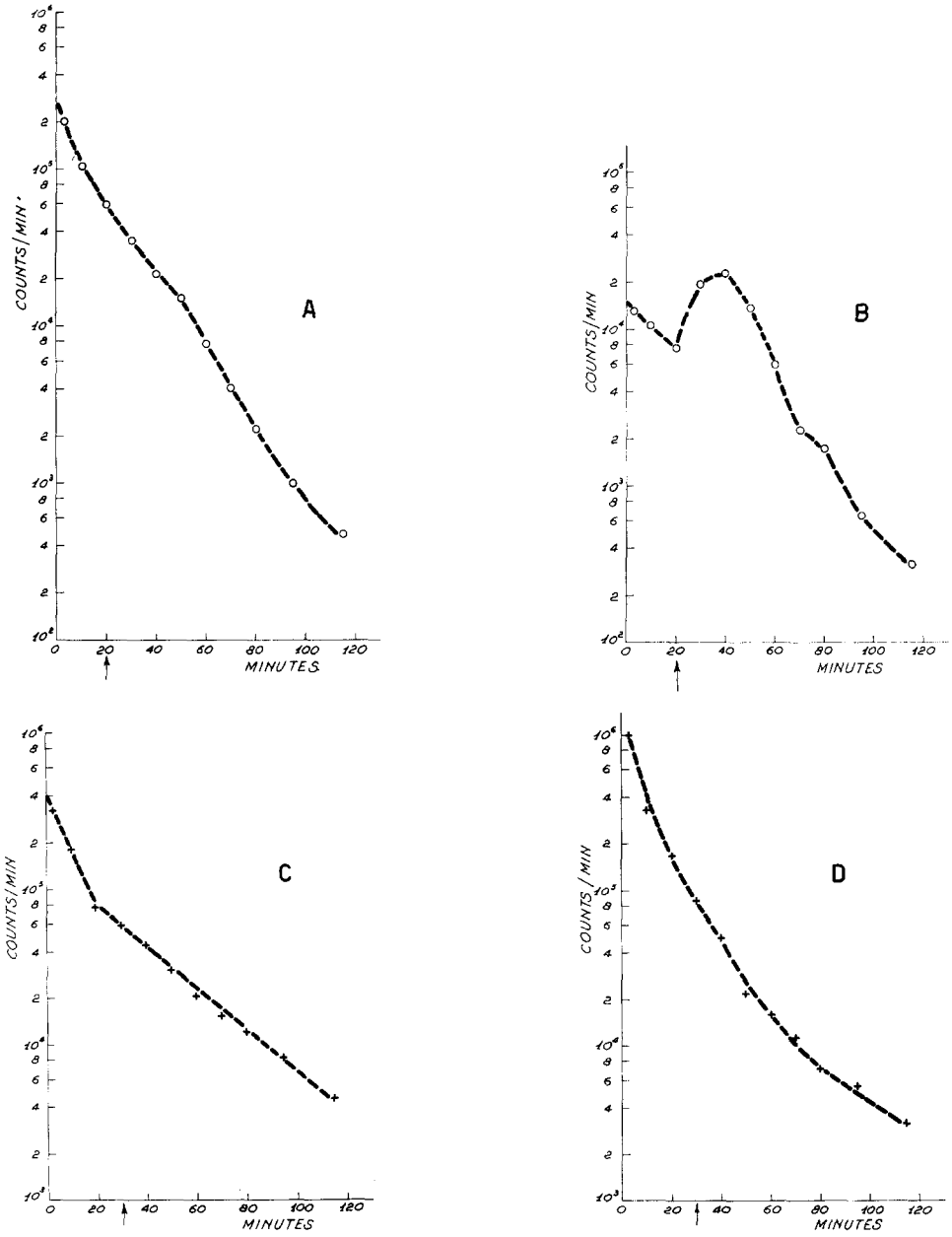

Fig. 2. Washing-out fluxes of thiourea in the small and large intestines. Every value is referred to a 10-min flux; the arrow indicates the addition of Amphotericin B (10 μg/ml). A, mucosal washing-out flux in the small intestine. B, mucosal washing-out flux in the large intestine. C, serosal washing-out flux in the small intestine. D, serosal washing-out flux in the large intestine.

across the large intestine. The effect was slight when compared to that occurring on thiourea.

Finally, Table IV shows that Amphotericin B did not significantly affect permeability across the small and large intestines of a substance soluble in lipid solvents, *i.e.* phenylacetic acid.

TABLE IV

PHENYLACETIC ACID EFFLUX ACROSS THE SMALL AND LARGE INTESTINES IN THE PRESENCE OF AMPHOTERICIN B IN THE MUCOSAL FLUID

Φ_A = phenylacetic acid efflux in the presence of Amphotericin B. Φ_C = phenylacetic acid efflux in control periods. The figures in parentheses represent the number of experiments.

Tissue	*System*		*Period*	Φ_0* (μmoles·cm^{-2}·h^{-1}·10^{-2})	$\frac{\Phi_A - \Phi_C}{\Phi_C} \times 100$**
Small intestine	Control	(4)	1st	4.1	
			2nd	3.6	
	Amphotericin B, 10 μg/ml	(4)	1st	3.6	− 4.3 ± 4.1
			2nd	3.5	− 7.2 ± 3.8
			3rd	3.3	−12.4 ± 6.0
			4th	3.0	−13.3 ± 7.3
Large intertine	Control	(4)	1st	4.1	
			2nd	3.9	
	Amphotericin B, 10 μg/ml	(4)	1st	3.8	− 0.8 ± 2.0
			2nd	4.0	+ 2.2 ± 3.7
			3rd	4.8	+23.2 ± 19.0
			4th	4.9	+23.1 ± 20.2

* Mean value.
** Mean value ±S.E.

DISCUSSION AND CONCLUSIONS

Permeability of some epithelial tissues to a typically water-soluble substance such as thiourea was differently affected by Amphotericin B. The action of polyene added to the mucosal fluid was stronger on the large than on the small intestine permeability. As newt, rat and guinea-pig small intestines are almost unaffected by Amphotericin B (C. LIPPE AND B. GIORDANA, unpublished observations), this phenomenon seems to be a general property of the small intestine.

According to VAN ZUTPHEN, VAN DEENEN AND KINSKY[5], artificial bilayer membranes with a 1:1 phospholipid–cholesterol molar ratio are more sensitive to the action of polyene antibiotics than membranes with a 10:1 ratio. In agreement with these results, a large intestine luminal membrane should be expected to show a phospholipid–sterol ratio lower than that found in small intestine brush border. We have no chemical data concerning the phospholipid–sterol ratio in large intestine luminal membranes, although in small intestine this value ranges from 2 (rat)[8] to 1 (guinea pig)[9]. A similar ratio has been found in erythrocyte membranes[9], which are very sensitive to polyene action. Hence, the relative insensitivity of the small intestine to polyene action must be accounted for by a factor other than the phospholipid–cholesterol ratio. It is well known[10] that intestinal microvilli have a high protein

content. This might prevent Amphotericin B action by a polyene–protein binding, as with serum proteins[11], or by an interaction of proteins with membrane lipids which could inhibit molecular rearrangements.

As for *Bufo marinus* bladder[3], when Amphotericin B is added to the serosal side, even at very high concentrations, no effect on thiourea permeability in the small and large intestines is observed. Since our intestinal epithelial preparations were previously stripped with the technique of BAILLIEN AND SCHOFFENIELS[6], this lack of effect can be hardly accounted for by a restraint to the diffusion of polyene across non-epithelial tissues. Hence it is possible that serosal membranes have a structure or composition different from mucosal ones.

Unlike monocellular organisms, epithelia are slightly modified by the action of polyenes. The electron microscopic studies of LICHTENSTEIN AND LEAF[3] appear to support the idea that Amphotericin B causes no deep alterations in *Bufo marinus* bladder. Our data seem to confirm these findings. In fact, small and large intestine permeability to phenylacetic acid was not appreciably modified after treatment with Amphotericin B. Deep modifications in epithelial barriers due to polyene action should result in a modified permeability to substances soluble in lipid solvents.

Furthermore Amphotericin B exhibits a weak effect on permeability to water-soluble substances with higher molecular weight than thiourea, such as creatinine. Deep disorganization in cell membranes should be expected to produce a similar modification in permeability to all water-soluble molecules.

Besides, these results confirm, in another system, that Amphotericin B is a 'weaker' polyene, which causes less extensive membrane damage than other 'stronger' polyenes as filipin[12].

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