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WEAK ACID ACCUMULATION IN THE SEROSAL EXTRACELLULAR COMPARTMENT OF THE FROG GASTRIC MUCOSA

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Summary

The dimethyloxazolidine dione distribution in the extracellular compartments of the frog gastric mucosa was analyzed by washout kinetics. The volumes of the two extracellular compartments, serosal and mucosal, were estimated by inulin washout as 0.435 ± 0.019 and 0.176 ± 0.018 μl/μl tissue water, respectively. In the serosal extracellular space, significant dimethyloxazolidine dione accumulations of 2.63 ± 0.25, 2.28 ± 0.16, and 1.86 ± 0.08 times that of the bathing media were found for bathing solutions with pH values of 6.9, 7.4, and 7.9 respectively. A high pH of the serosal extracellular fluid by itself could not account for the high values of dimethyloxazolidine dione accumulation. A difference in the total dimethyloxazolidine dione accumulation requires: (a) the existence of differences in the pH values and also the existence of a difference in the diffusion coefficient of the two forms of dimethyloxazolidine dione; or (b), a binding of one of the two forms, i.e., binding of dimethyloxazolidine dione form by fixed charges.

Introduction

The frog gastric mucosa includes the continuous cell layer that covers the surface, the pits and the tubules. In the tubules are found the microvilli projecting into the lumen of the gastric gland [1] and the vesicotubules in the apical part of the oxyntic cell cytoplasm [2]. At the serosal side, there are muscularis mucosae, part of the submucosa [3] and the intercellular spaces between the interdigitated lateral cell membranes [4].

It has been proposed that the H^+ concentration in these complex extracellular compartments facing the serosal and mucosal surfaces differs from those of the bathing solutions. In the serosal extracellular space, the pH can increase due to a substantial reduction in the CO_2 concentration [5] and to an accumulation

of HCO_3 which occurs during H^+ secretion [6]. Conversely, at the mucosal side, the pH is expected to be lower immediately outside the cells than at the bathing solution. This should be so since it is well established that primary secretion accumulates in the extracellular compartment before it reaches the less acid mucosal bathing solution.

It seems from these considerations that, the existence of differences in pH, between the extracellualr compartments and the bathing media, can produce in the serosal and mucosal extracellular compartmenst different ratios of the ionized to the un-ionized forms of a weak acid placed in the bathing solutions. Parenthetically, in this situation, attempts to measure intracellular pH with a weak acid are invalidated [7] [e.g., 5,5-dimethyloxazolidine-2,4-dione vide infra].

On the other hand, it seems possible to use the kinetics of the distribution of a weak acid to measure its activity in the extracellular compartments of the frog gastric mucosa, specially at the serosal surface, where we previously proposed the existence of a compartment with diffusion properties different to those of an aqueous solution [8].

For this purpose, washout kinetics of dimethyloxazolidine dione were used in order to estimate its distribution in the extracellular compartments of the frog gastric mucosa. The extracellular compartment volumes were estimated by inulin washout.

Methods

Frogs (*Rana pipiens)* used in these experiments were pithed, their stomachs removed and opened along the small curvature and the mucosa stripped by blunt dissection.

Composition of the solutions

Three groups of experiments were performed using solutions with pH values of 6.9, 7.4, and 7.9. The solutions used in the experiments were basically those previously described. The 7.4 pH buffered solution has the following composition [9] in mM: NaCl, 86.6/KCl, 3.2/$CaCl_2$, 1.8/KH_2PO_4, 0.8/$MgSO_4$, 0.8/$NaHCO_3$, 17.8/glucose 22.0. The $NaHCO_3$ concentration of this solution was changed to 5.8 and 57.5 mM to prepare the 6.9 and 7.9 pH buffered solutions, respectively. In both cases, the NaCl was changed to keep the Na^+ concentration constant. The osmolality of the solutions was between 218 and 225 mosM/kg water. All the solutions were bubbled with 95 : 5%, O_2/CO_2. The $NaHCO_3$/5% CO_2 buffer can be used within the range of 6.0 to 8.0. The desired pH of the solution can be adjusted by changing the concentration of $NaHCO_3$ in equilibrium with the gas phase containing 5% CO_2 [10]. The pH of all the solutions used to load and wash the mucosa was always checked before and after use. Histamine diphosphate was added to a final concentration of 10^{-4} M.

Experiments to measure dimethyloxazolidine dione and inulin washout

The mucosae were loaded for 90 min in buffered solutions labelled simultaneously with [^{3}H]inulin and 5.5-[^{14}C]dimethyloxazolidine-2,4-dione. After blotting, each mucosa was then mounted as a flat sheet between two symmetri-

cal chambers. The volume of each chamber was 4 ml. The exposed area of the preparation was 1.13 cm^2. Both chambers were filled with buffered, unlabelled solution of the same pH used to load the mucosa. The solutions in the chambers were totally replaced at times 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 min. Finally, the mucosa was weighed and extracted during 48 h at 40°C in 10 ml of the same buffered solution. Dry weight was obtained after extraction.

^3H and ^{14}C activities were counted in samples of: (a) 0.01 ml of the loading (labelled) solution plus 1 ml of the unlabelled solution; (b) 1 ml of each of the washing solutions; (c) 1 ml of the tissue extract. In order to prevent quenching differences due to protein content, 0.1 ml of an extract of fresh mucosa in 1 ml of unlabelled buffered solution was added to each of the samples (a) and (b). All samples were counted with 10 ml Instagel. Total water content was obtained from the wet and dry weights and found to be 0.85 of the wet weight, confirming previous results [11].

Results

Fig. 1 shows the results of the dimethyloxazolidine dione washout from 10 mucosae with the 6.9 pH solution on both sides. The ordinates present the quotient of the activity in the tissue water remaining in the tissue divided by the activity in loading solution. The activity remaining in the tissue was obtained by adding the activities recovered in the solutions placed in the chambers after the corresponding time and divided by the tissue water volume. Each point is the mean ± standard error. All values (a total of 110, i.e., 10 mucosae times 11 determinations) were fitted by a 2-exponential curve. (Equation given in the figure). Similar experiments were performed with 7.4 pH solution on both sides, and 7.9 pH solution on both sides in two groups of 10 experiments each. Washout curves were treated similarly to that presented in Fig. 1 and the results are given in Figs. 2 and 3. The 2-exponential model was chosen because in a 3-exponential model previously used [12], the third compartment represented less than 0.005 of the total activity in the mucosa.

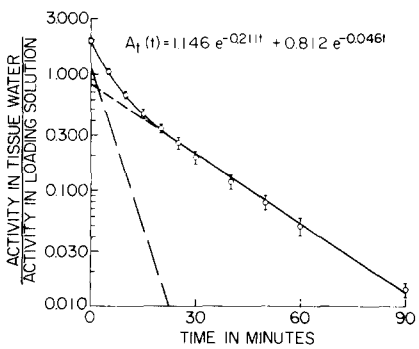

Fig. 1. Efflux of dimethyloxazolidine dione from mucosae at pH 6.9. The ln's mean ±SE of the activities remaining in the tissue are presented as function of the washout time. Values are fitted by 2 exponential curves when the activity of the extract (less than 1% of the initial activity in the tissue) is discarded. The equation of the curve is also shown in the figure.

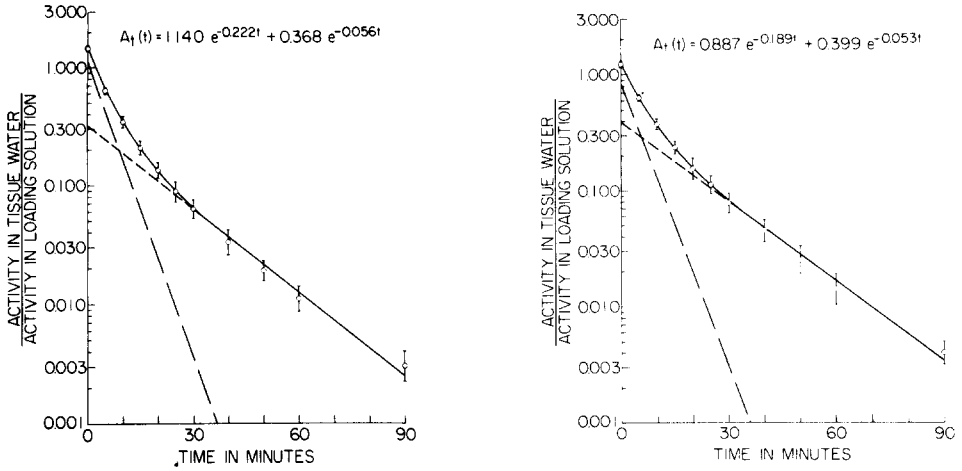

Fig. 2. Efflux of dimethyloxazolidine dione from mucosae at pH 7.4. The ln's mean ±SE of the activities remaining in the tissue are presented as function of the washout time. Values are fitted by 2 exponential curves when the activity of the extract (less than 1% of the initial activity in the tissue) is discarded. The equation of the curve is also shown in the figure.

Fig.3. Efflux of dimethyloxazolidine dione from mucosae at pH 7.9. The ln's mean ±SE of the activities remaining in the tissue are presented as function of the washout time. Values are fitted by 2 exponential curves when the activity of the extract (less than 1% of the initial activity in the tissue) is discarded. The equation of the curve is also shown in the figure.

The values obtained for exponents λ_1 and λ_2 and for the coefficients A and B in the three groups of experiments are presented in Table I. The difference between λ_1 and λ_2 is highly significant ($P < 0.001$) in the three cases. The half time of the rapid phase (ln 2/λ) of the dimethyloxazolidine dione (M_r = 129) washout, calculated from column 2, are 3.29 ± 0.02, 3.12 ± 0.01, and 3.67 ± 0.02 for solutions with pH values of 6.9, 7.4, and 7.9, respectively. These values are close to the 3.56 ± 0.12 min previously reported for the extracellular Cl^- (equiv. wt. = 36) efflux [9]. This agreement in the time of washout, even considering the difference in weight, suggests that the rapid phase of dimethyloxazolidine dione washout represents mainly the extracellular clearance. The slow phase of the washout may consequently represent the contribution of the

TABLE I

WASHOUT OF [^{14}C]DIMETHYLOXAZOLIDINE DIONE AS FUNCTION OF THE SOLUTIONS' pH, DESCRIBED BY TWO EXPONENTIAL CURVES IN WHICH λ_1, λ_2 TIMES *t* ARE THE e EXPONENTS AND *A* AND *B* ARE THE COEFFICIENTS

(1) pH of the solutions	(2) λ_1 min^{-1}	(3) λ_2 min^{-1}	(4) *A* cpm/μl tissue water / cpm/μl loading solution	(5) *B*
6.9	—0.211 ± 0.001	—0.046 ± 0.001	1.146(1.153—1.139)	0.812(0.852—0.774)
7.4	—0.222 ± 0.001	—0.056 ± 0.008	1.140(1.166—1.114)	0.368(0.433—0.313)
7.9	—0.189 ± 0.001	—0.053 ± 0.002	0.887(0.893—0.881)	0.399(0.447—0.356)

pooled cell compartment. Accordingly, in the treatment of these values, the following three-compartment-series model is proposed: compartment 1 is formed by the solutions bathing both serosal and mucosal surfaces (8 ml changed 11 times during 120 min). Compartment 2 is formed by the serosal and mucosal extracellular spaces. These two compartments, even though independent, were added together in this treatment (dimethyloxazolidine dione$_2$) (DMO_2). Compartment 3 is the cellular compartment.

The dimethyloxazolidine dione activity in the cellular compartment (DMO_3) at $t = 0$ was estimated using an equation from the appendix by A.F. Huxley in a paper by A.K. Solomon [13], and using the values reported in Table I for λ_1, λ_2, A and B.

$$DMO_3 = \frac{A \cdot B(\lambda_1 - \lambda_2)^2}{A \cdot \lambda_1^2 + B \cdot \lambda_2^2}$$

The values obtained from these calculations are presented in the second column of Table II.

The activities of dimethyloxazolidine dione recovered across each surface by the total dimethyloxazolidine dione recovered from the cellular compartment are given in columns 3 and 4 of Table II. From the values of λ_1, it is evident that after 30 min more than 99.7% of the dimethyloxazolidine dione$_2$ has been washed out. After that time the dimethyloxazolidine dione efflux comes mainly from the cellular compartment. The last 2 columns of Table II present the products obtained by multiplying column 2 by columns 3 and 4 respectively.

Columns 2 and 3 of Table III shows the activities of the mucosa,compartment 3, recovered through each surface. Once these 2 values were obtained, the contributions from compartment 3 shown in the last 2 columns of Table II were subtracted from them to obtain the initial activity ($t = 0$) of the two extracellular compartments: $DMO_2^{serosal}$ and $DMO_2^{mucosal}$. The units are the dimethyloxazolidine dione activity in each compartment per μl tissue water divided by the activity per μl loading solution.

However, it is necessary to estimate the water volume of these extracellular compartments to evaluate the real accumulation of dimethyloxazolidine dione

TABLE II

WASHOUT OF DIMETHYLOXAZOLIDINE DIONE FROM THE CELLULAR COMPARTMENT

(1) pH of the solutions	(2) DMO_3 cpm/μl tissue water / cpm/μl solution	(3) $\frac{DMO_{80-90}^{serosal}}{DMO_{80-90}^{total}}$	(4) $\frac{DMO_{80-90}^{mucosal}}{DMO_{80-90}^{total}}$	(5) $DMO_3^{serosal}$ cpm/μl tissue water / cpm/μl solutions	(6) $DMO_3^{mucosal}$
6.9	0.480 ± 0.023	0.847 ± 0.014	0.153 ± 0.014	0.407 ± 0.021	0.073 ± 0.008
7.4	0.208 ± 0.029	0.865 ± 0.010	0.135 ± 0.010	0.180 ± 0.025	0.028 ± 0.004
7.9	0.202 ± 0.022	0.812 ± 0.011	0.188 ± 0.011	0.164 ± 0.018	0.038 ± 0.005

TABLE III

EXTRACELLULAR DIMETHYLOXAZOLIDINE DIONE ACTIVITY

(1) pH of the solutions	(2) Activity recovered cpm/μl tissue water / cpm/μl solution	(3)	(4) Extracellular activity cpm/μl tissue water / cpm/μl solution	(5)	(6) Extracellular activity cpm/μl extracellular water / cpm/μl solution	(7)
	Serosal surface	Mucosal surface	Serosal surface	Mucosal surface	Serosal surface	Mucosal surface
6.9	1.552 ± 0.094	0.400 ± 0.031	1.145 ± 0.096	0.327 ± 0.032	2.632 ± 0.249	1.858 ± 0.263
7.4	1.171 ± 0.049	0.291 ± 0.013	0.991 ± 0.055	0.263 ± 0.014	2.278 ± 0.161	1.494 ± 0.172
7.9	0.972 ± 0.046	0.266 ± 0.034	0.808 ± 0.049	0.228 ± 0.034	1.857 ± 0.075	1.295 ± 0.234

with respect to the specific activity of the loading solution.

As was explained in Methods, inulin spaces were determined in the same mucosa used for the washout of the dimethyloxazolidine dione. The inulin spaces correspond to 0.435 ± 0.019 and 0.176 ± 0.018 of the total tissue water for the serosal and for the mucosal surface respectively. In these calculations, we consider that inulin specific activity in the extracellular compartments is the same as that obtained in the loading solution.

Discussion

The difference proposed in the literature between the HCO_3^- and the H^+ concentrations in the serosal and in the mucosal extracellular spaces and the bathing media are based on indirect observations. The bases for the proposed lowering of CO_2 activity in the serosal compartment is the requirement for exogenous CO_2 during acid secretion [5,14]. The proposed increment in concentration at the serosal cell border is also based in the requirement to dissipate the OH^- neutralized by CO_2 in the cellular compartment [6]. The accumulation of the primary acid secretion at the mucosal surface [15] has not been directly measured.

The experiments reported in this paper are intended to provide information on the dimethyloxazolidine dione distribution on these extracellular compartments.

The first difficulty in achieving this purpose is the measurement and identification of these extracellular compartments. The possibility that the spaces reached by inulin from each surface could be used for this purpose requires: (a) that labelled inulin not be bound or adsorbed by the tissue; (b) that the inulin remain excluded from the cellular fraction; and (c) that the inulin does not cross the mucosa during the washout.

The possibility that inulin is bound or adsorbed to some tissue fraction seems unlikely because the measured inulin spaces are independent of the specific activity (within 2 orders of magnitude) of the labelled inulin used. This indicates that the presence of the unlabelled inulin has no effect in the tracer distribution.

Concerning the possibility that inulin penetrates the cellular fraction, Page [16] has shown, for cat papillary muscle, a rapid entry of inulin to equilibrate with 24% of the total water in 1 h, followed by a continuous but very slow increase, suggesting the existence of two spaces to be reached by inulin. McIver and Macknight [17] have also presented evidence of 2 volumes measured by inulin diffusion, when the tissue is extracted before or after drying. This effect of drying was observed in kidney and liver slices from rabbit and guinea pig. However, no significant difference was found for toad-bladder epithelial cells. In the frog gastric mucosa no significant ($P > 0.30$) difference is observed between the inulin volume of 0.611 ± 0.026 of the total water obtained in the washout experiments reported here, and the 0.560 ± 0.045 of the total water reported in mucosae previously dried [11]. This suggests that the possible existence of a second inulin compartment may be disregarded in the frog gastric mucosa.

With respect to the inulin transmucosal flux, the value obtained for the ratio

of the effective area for diffusion to the thickness of the mucosa ($A/\Delta x$) is 0.04 ± 0.06 cm. This value is not significantly different ($P > 0.40$) from zero [18]. The values obtained when the mucosa was simultaneously labelled in both surfaces were used as a better approach to avoid the possible error due to small transmucosal flux that may occur.

Dividing the dimethyloxazolidine dione extracellular activity reported in Table III (cpm per μl tissue water/cpm μl loading solution) by the inulin volume (μl extracellular volume/μl tissue water) the ratios of the activities in the extracellular spaces to that in the loading solution were obtained (cpm per μl extracellular volume/cpm μl loading solution). These values are presented in the last two columns of Table III.

The total activity of the dimethyloxazolidine dione should be equal in the extracellular compartment and in the loading solution when it diffuses freely in the unionized forms, even in the case of both compartments having different pH values. In which case, the ratios, ionized to un-ionized forms in the two compartments, differ according to the compartment's pH values. If this were so, it would be impossible to detect the H^+ or HCO_3^- accumulation.

In the case of only the un-ionized form of the weak acid being able to diffuse between the two compartments, the pH of 1 compartment can be estimated knowing the pH of the other compartment and the ratio between the 2 total concentrations [7].

Between these two extreme cases, free diffusion and total restriction to the ionized form of the weak acid, the estimation of a pH values is invalidated, even when total activities are different in both compartments.

Consequently, an equal total concentration in the two compartments would not prove that the pH values are the same. On the contrary, a difference in the total dimethyloxazolidine dione accumulation requires: (a) the existence of differences in the pH values and also the existence of a difference in the diffusion coefficients of the 2 forms of dimethyloxazolidine dione, and/or (b) a binding of 1 of the 2 forms, i.e., binding of dimethyloxazolidine dione$^-$ form by fixed charges.

In the case of the mucosal extracellular space the ratio $DMO_2^{mucosal}/DMO^{sol}$ is not always different from one ($P > 0.05$). Since the mucosal extracellular compartment is more acid than the solution, the possibility of getting higher total dimethyloxazolidine dione activities than in the solution by effect of pH is excluded. Consequently pH by itself is unable to explain the experiments in which the dimethyloxazolidine dione accumulates in this compartment.

In the case of the serosal extracellular compartment, which is more alkaline than the solution, the ratio $DMO_2^{serosal}/DMO^{sol}$ is always significantly higher than one ($P < 0.001$). Here pH differences may account for this accumulation, but either one or both of the above possibilities, pH or binding, may prevail. Independently of which possibility prevails, the fact is that the dimethyloxazolidine dione accumulates in the serosal extracellular compartment.

In short, without excluding the possible effect of pH differences in the dimethyloxazolidine dione distribution in the extracellular space, the presence of some binding in this space is required, at least in the cases of accumulation in the extracellular mucosal surface.

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