TRANSPORT OF TRITIATED WATER ACROSS ISOLATED GUT OF FRESHWATER CATFISH, *CLARIAS GARIEPINUS*: THE EFFECTS OF SODIUM FREE RINGER AND HYPOOSMOTIC SALINE

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ABSTRACT

The movement of tritiated water through the isolated, perfused gut tissue of the catfish, *Clarias gariepinus* (lazera), was studied at constant perfusion rate.

The gut epithelium, the external medium and the perfusate constituted a system of three compartments in series for water exchange. This allowed for the determination of the characteristics of tritiated water movement through the apical and basal barriers.

Perfusion of the gut epithelium with Na^+-free Ringer and hypoosmotic saline produced substantial reduction in the permeability constants of both barriers and the rate at which the tissue reached equilibrium with respect to tracer flux. The reduction due to hypoosmotic saline was dependent on concentration gradient of the saline. The lower the concentration gradient, the less the reduction.

INTRODUCTION

The study of teleost gut has shown that the epithelium is the site of considerable salt and water diffusional fluxes (Smith, 1930; Utida *et. al.*, 1967). An understanding of the nature of fluxes across the gut epithelium would necessitate a direct access to the bathing media on both serosal and mucosal side of the membrane. An application of the technique of isolated, perfused head of trout (Payan and Matty, 1975) modified by Girard and Payan, (1976) to the isolated gut allows for the determination of the relative permeabilities of the mucosal (apical) and serosal (basal) barriers from the kinetics of the radioactive loading and unloading of the gut epithelium.

Previous studies on teleost gut have shown that the gut epithelium displays adaptive functional features in relation to salinity changes. Increased salinity causes increased water absorption in isolated eel gut (Utida *et. al.*, 1967). Serosal hypertonicity in anuran urinary bladder causes increased water permeability across epithelial membrane (Hardy, 1979). Increased water absorption has also been reported to increase net Na^+ uptake (Oide, 1967). Thus, water absorption by the gut of teleosts is solute-linked and responds to changes in external salinity.

The cellular compartment of the gut epithelium is limited by two barriers, the apical and the basal. Both have different permeability characteristics for water, Na^+ and Cl^- movement (Girard and Payan, 1977). This study was designed to examine the relative permeabilities of both barriers in *C. gariepinus* to tritiated water. The effects of Na^+-free Ringer and hypoosmotic saline solutions were examined.

MATERIALS AND METHODS

Adult *C. gariepinus* of either sex and weighing between 200-350g were used in this study. They were obtained from Oyo State Government fish pond at Ogbomoso, Nigeria. They were maintained and aclimatized for at least one week in large outdoor (23+1°C) tanks arranged in a cascade with a flow-through water supply. They were fed on commercial fish pellet, but this was discontinued three days before an experiment. All experiments were carried out in the laboratory at ambient temperature (24+1°C).

The fish were stunned with a sharp blow to the head and the gut exposed by a midline incision of the ventral muscle in the abdominal region. The exposed gut was kept moist with Ringer's solution (Bergman, *et. al.*, 1974). Gut was severed just posterior to the pyloric sphincter and flushed in-situ with 15ml of Ringer's solution to clear the materials in the gut lumen.

A flarred afferent polythene catheter (Intramedic PE90) was inserted into the anterior and posterior openings of the intestine and held in place with a purse-string ligature using a nylon thread. Pre-incubation was carried out for 30 minutes and the gut was perfused at the rate of 3.05+0.05ml min^{-1} using a Watson-Marlow peristaltic flow inducer pump. Ringer's solution was prepared according to Bergman *et. al.* (1974) except that O.01M Tris buffer was used to adjust the pH to 7.4 and 0.9gl^{-1} glucose added just prior to use. Na+-free Ringer was prepared by substituting choline chloride for sodium salts.

To an organ bath containing 30ml of cold Ringer's solution. 10 ul of tritiated water (25,OmC) was injected using Hamilton microliter syringe. An initial 200,ul sample of the bath was taken before the start of the experiment to obtain total activity within the bath. After pre-incubation period, the gut was transferred into an organ bath containing the hot Ringer; and effluent collection started immediately. In the first 5 minutes, 200,ul aliquots were taken every 30 seconds and discharged into a scintillation vial. After 30 minutes, subsequent sampling was done every 15 minutes for 2½ hours as test runs had demonstrated that the gut remained viable for longer than 2½ hours after isolation. The serosal bathing fluid was sampled at the end of the experiment after the removal of the gut.

All the samples were counted on the Packard Tris-Carb liquid scintillation spectrometer model C2425 after the addition of 5ml of Bray's solution (Bray, 1960).

The gut epithelium was considered to behave as a single compartment (2) bound by different barriers for THO diffusion towards the outer (1) and the inner (3) external media. This gave a system of three compartments in series with four rate constants, k_{12}, k_{21}, k_{23} and k_{32} (Fig. 1).

Calculation of the constants were as in Clarkson and Linderman, (1969); Girard and Payan (1977); Kusemiju and Oduleye (1989). The present method did not permit the evaluation of K_{32} either directly or indirectly because it had the advantage of limiting backflux which could introduce error into the computation of rate constant of all the barriers.

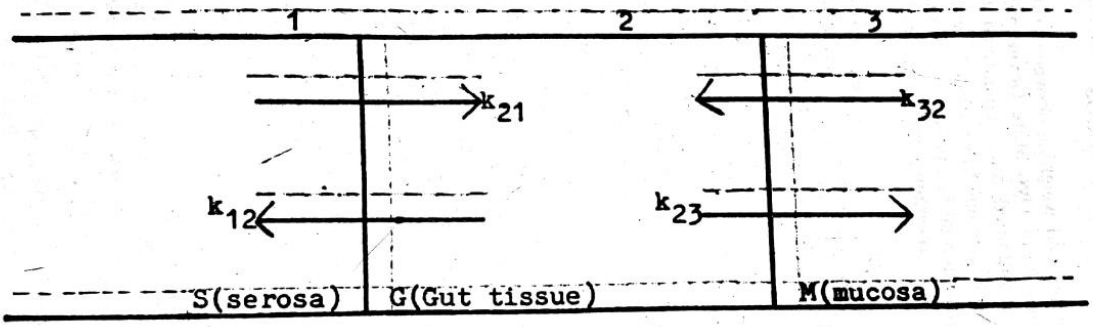


Fig. 1: Model of three compartment system (example of transfer coefficients, k_{21} = fraction of water in compartment 2 renewed per minute across the membrane from 2 to 1) (Girard and Payan, 1977).

RESULTS

The results of the experiments conducted showed that serosal-to-mucosal fluxes reached a steady state within two minutes as O was found to be 1.57 minute (Fig. 2). O representes the time it takes for the tissue to reach equilibrium with respect to tracer flux. This was obtained by plotting the cumulative appearance of radioactivity in internal compartment against time. O was determined by the point of intersection of the asymptote of the curve with the t axis. O values were also determined for Na^+-free Ringer and hypoosmotic saline. Absence of Na^+ in the perfusate produced 16% increase in O value (1.82+O.9min) (Fig. 3). Perfusion of the gut with hypoosmotic saline whose strength were 10, 25, 50 and 75% of normal Ringer's solution produced 2.25, 1.74, 1.64 and 1.32 O values respectively (Fig. 4). There was thus substantial decrease in O value with decreased saline concentration.

From the O values, calculated permeability constants are shown for Na^+ free Ringer (Table 1) and hypoosmotic saline (Table 2). Na^+ absence produced 29, 39 and 67% decrease for k_{12}, k_{21} and k_{23} respectively. High percentage decrease in the rate constant (67%)

Table 1: Effect of Na^+-free Ringer on the permeability of apical and basal barriers in *Clarias gariepinus*.

	Control (8)	Na^+-free Ringer (8)	% Difference
O	1.57+0.18	1.82+0.19	16.0
k_{12}	0.041+0.004	0.029+0.007	−29.3
k_{12}	0.77+0.12	0.47+0.045	−39.0
k_{23}	0.043+0.007	0.014+0.003	−67.3

Values are Mean+SE of eight replicates.

Table 2: Effects of hypoosmotic saline on the permeability of the apical and basal barriers in *Clarias gariepinus*.

	Control (8)	75% Ringer (8)	50% Ringer (8)	25% Ringer (8)	10% Ringer (8)
O	1.5+0.18	1.32+0.07	1.64+0.10	1.74+0.09	2.29+0.06
*%		- 16	+ 45	+ 11	+ 48
k_{12}	0.041+0.004	0.037+0.006	0.036+0.004	0.027+0.003	0.022+0.002
%		- 10	- 12	- 34	- 46
k_{21}	0.77+0.12	0.68+0.02	0.56+0.03	0.51+0.03	0.043+0.02
%		- 12	- 27	- 34	- 44
k_{23}	0.043+0.007	0.04+0.008	0.04+0.007	0.034+0.004	0.03+0.003
%		- 7	- 7	- 21	- 28

Values are Mean+SE of eight replicates.

*% difference from control value.

showed that the effect was more significant in the cellular compartment.

The same trend was observed with the hypoosmotic saline. The decrease was however, not significant at 75 and 50% saline concentrations. For these two concentrations, the k_{23} values were - 7% different from the normal Ringer. Thus, the effect of the hypoosmotic saline was more felt in the apical than the basal barrier.

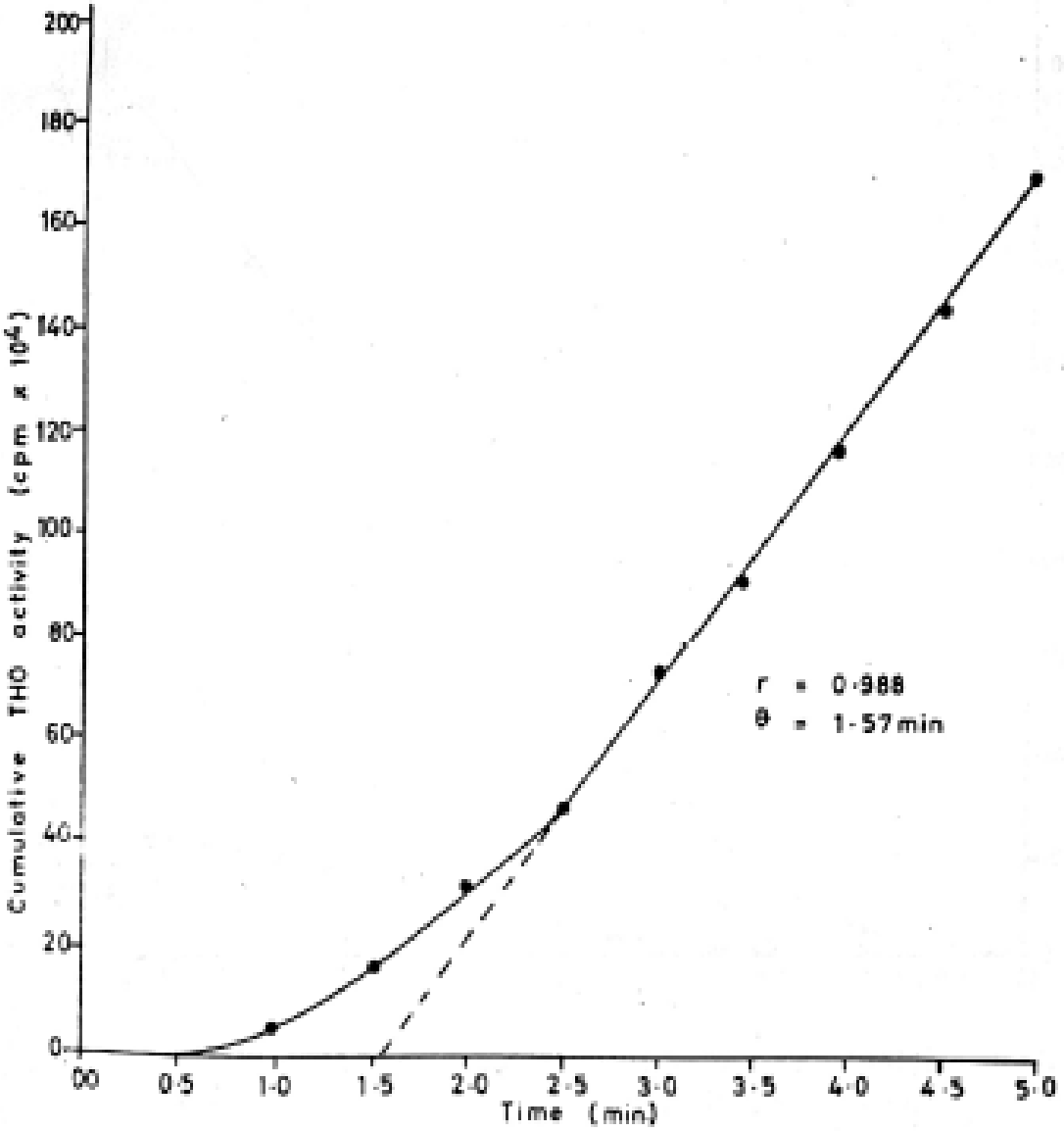
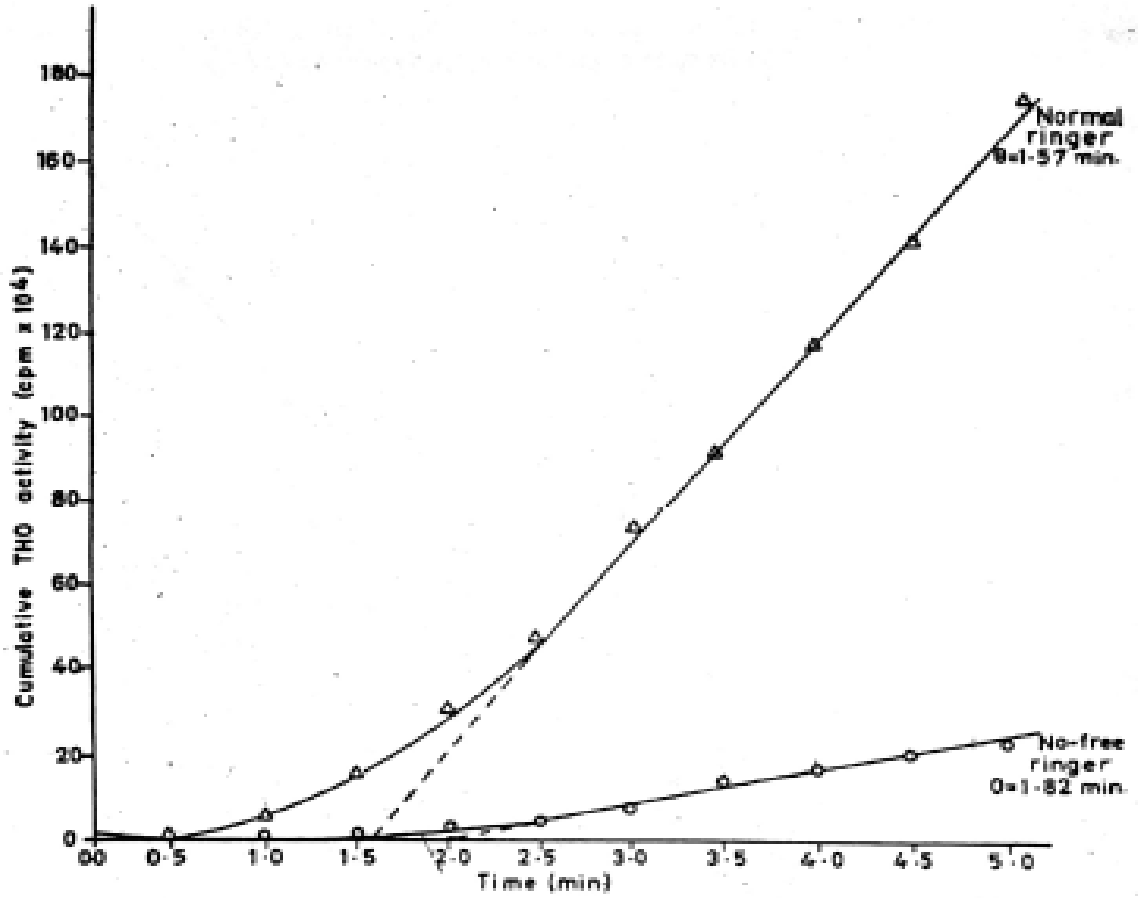


Fig 2 Cumulative appearance of THO in the luminal compartment of C. gariepinus



Fig·3 Effect of Na^+—free Ringer on rate of appearance of THO in the luminal compartment of *C. gariepinus*

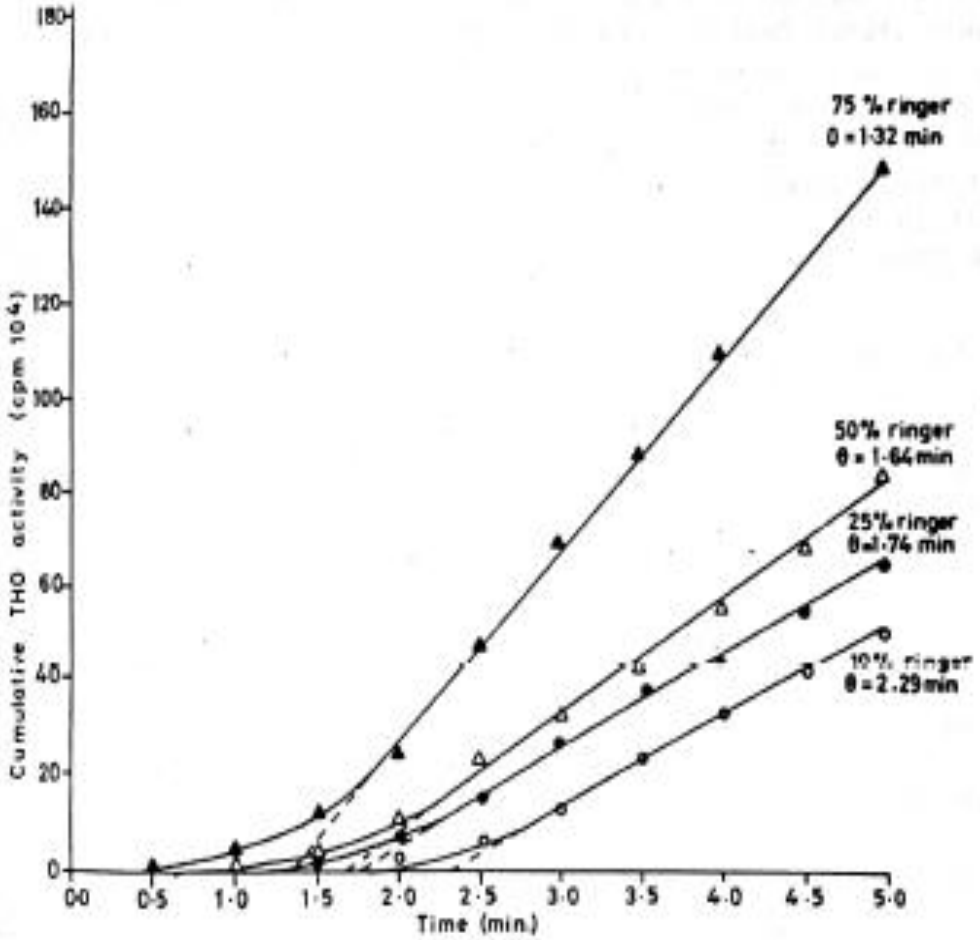


Fig. 4 : Effect of hypoosmotic saline on rate of appearance of THO in luminal compartment of *C. gariepinus*

DISCUSSION

Either of the apical and basal membranes could constitute the limiting barrier in the transport of THO across the gut epithelium of *Clarias gariepinus*. For normal Ringer, turn-over rate of THO in the intermidiate compartment was about 11 times more rapid across the apical than the basal barrier (Kuku and Oduleye, (1989)' Thus, the latter constitutes the limiting barrier to water (T_2O) movement across the gut epithelium in *C. gariepinus*. In the branchial epithelium of *Salmo gairdneri*, movement across the apical membrane is seven times more rapid than across the basal barrier (Isaia *et. al.*, 1978). In toad urinary bladder, the apical membrane is the limiting barrier to water diffusion (Parish and Piccini, 1973). In this respect, the gut epithelium of C.gariepinus is similar to the branchial epithelium of S. gaildneri and differed from the toad urinary bladder.

Na^+ -free Ringer solution produced a turn-over rate of THO which was 20 times more rapid across the apical than the basal barrier (Table 1). This suggests that water permeates the apical gut epithelium at a slower rate in the absence of Na^+. In the absence of Na^+ , the tissue reached equillibrium with respect to tracer flux at a slower rate.

Hypoosmotic saline of different concentrations has similar effect on the tissue (Fig.4). Present study also shows that decreased concentration gradient causes increase in permeability constant. The fundamental change in the Ringer's solution was in relation to the concentration of ions. Hardy (1979) showed that serosal hypertonicity induced an increase in water permeability in anuran urinary bladder. The intracellular Ca^{++} and Na^+ were known to play central role. The result of the present study is consistent with reports that water transport across membrane barriers are solute-linked (Oide, 1967: Potts *et. al.*, 1967; et. *al.*, 1973).

Cations are critical in induction of osmotic water flow in toad bladder (Hardy, 1979). Deletion of Na^+ and Ca^{++} from extracellular fluid decreased the intracellular concentration of both cations (Borle' 1978). In C. gariepinus, the high decrease in the permaebility constant across the basal membrane might not be unconnected with decrease in cell volume and absence of Na^+ in the perfusate. Sodium ion is a known intracellular messenger in such movement.

The fundamental change in the hypoosmotic saline is in relation to the concentration of ions, the same was applicable to the Na^+ - free Ringer. It can then be inferred that transport of water across the barriers is soluted-linked

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