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Metabolism of Depleted Turtle Bladder

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Summary. Oxygen consumption, glycogen content, transmural potential difference (PD), and short-circuit current (SCC) were measured in fresh turtle hemibladder sacs and in matching sacs depleted by 18 hr of incubation in aerated, substrate-free Ringer's solution. Percent of original values after depletion were: oxygen consumption, 61%; glycogen content, 36%; PD, 28%; SCC, 19%. PD and SCC responded to addition of substrate (5.5 mM glucose plus 2 mM pyruvate) by a lag period of approximately 2 hr followed by progressive increases lasting for many hours. Other experiments utilized split bladders. The epithelium and adhering connective tissue (mucosal fraction) were separated from the underlying smooth muscle and connective tissue (serosal fraction) and the oxygen consumption and glycogen content of slices of the two fractions determined. Mucosal oxygen consumption declined to 48% of the original value during depletion while serosal oxygen consumption (initially much lower than mucosal) was well-maintained at 95% of the original value. Substantial net synthesis of glycogen took place in both fractions of depleted bladders after addition of substrate. The ratio of moles of oxygen consumed to moles of glucose (from glycogen) disappearing during the 18-hr depletion period was approximately 5.5 for serosal tissue and within the range 30 to 61 for mucosal tissue. The mucosal ratio was incompatible with the utilization of glycogen as the major endogenous substrate during depletion under aerobic conditions. It is suggested that the oxidation of lipid supports most of the endogenous metabolism in the mucosal tissue of the turtle bladder.

Tissues depleted of endogenous hormones and substrates by long incubation in minimal media have been found to be useful in characterizing transport processes [6, 28, 29]. Such tissues are assumed to use up or lose endogenous reserves and to become sensitive to the effects of added hormones or substrates. The isolated urinary bladder of the water turtle, a tissue known to carry on vigorous ion transport [9, 10, 15, 31], shows good viability under depletion conditions [17]. To evaluate changes taking place in turtle bladders during depletion, glycogen levels, respiration rates, short-circuit

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current (SCC), and transmural potential difference (PD) of depleted and nondepleted bladders were investigated. The recovery of the same parameters after the addition of substrate to depleted bladders was followed. The findings and their implications for the characterization of the energy metabolism of the smooth muscle and epithelial components of the bladder are the subjects of the present paper.

Materials and Methods

Turtles *(Pseudemys scripta)* were obtained from Lemberger Supply Co., Oskosh, Wisconsin. They were kept in tanks with access to fresh running water but without feeding during the week or two between receipt and use.

Most experiments utilized bladders incubated as hemibladder sacs. Hemibladders were rinsed, mounted on glass cannulae, filled with approximately 10 ml of Ringer's solution, and placed in continuously aerated Ringer's solution. Depleted bladders were incubated for 18 hr. A histological study *(paper in preparation)* showed that depleted bladders retained structural integrity during this period.

Oxygen consumption was measured polarographically from slices measuring approximately 1 mm in the largest dimension. Instrumentation and procedure have been described elsewhere [16, 19, 21]. Temperature was constant at 25 °C. After a half-hour equilibration of slices within the respirometer vessel, oxygen consumption measurements were begun. In experiments requiring respiration rates for a one-hour period, three 20-min periods of observation, each beginning with a solution change, made up the hour. The first 5 to 7 min of each period were disregarded in calculating the rate of oxygen consumption to avoid error due to diffusional loss of oxygen from medium into tissue [21]. The mean rate calculated from the acceptable portions of the three periods was taken as the rate for the hour. As described previously, the spontaneous decline in oxygen consumption after the equilibration period was small [19].

Since the experiments to be described involve both sacs and slices, the question arises as to whether the oxygen consumption of slices is comparable to that of sacs. This question has been considered for tissues in general by Baldwin [1], and for the toad bladder in particular by Parisi and Bentley [25]. The latter authors examined oxygen consumption of bladder pieces of unspecified size and of bladders in two states of distension. They found that the respiration of well-filled sacs (comparable in distension to our turtle bladder sacs) exceeded that of pieces by 20%. In view of the uncertainties inherent in comparisons of oxygen uptake in different systems, this agreement is considered to be close. On the basis of Parisi and Bentley's findings, it is assumed that the oxygen consumption of turtle bladder slices reflects that of sacs to within 20%.

Some experiments involved the determination of oxygen consumption of mucosal (epithelial) and serosal (smooth muscle) components of the bladder. For this, bladders were dissected into mucosal and serosal fractions as described previously [19, 20, 24] and the two fractions utilized separately. The major components of the bladder wall and their proportions have been defined [19]. They are (a) mucosal fraction: epithelial cells, 1; connective tissue, 1, and (b) serosal fraction: smooth muscle, 2.3; connective tissue, 1.4.

Glycogen was determined by the Good, Kramer and Somygyi method [4, 11, 18] utilizing 0.2 to 0.5 g of tissue.

SCC and PD were determined on sacs following the methods of Bentley [3] and Walser [32]. Balanced calomel electrodes were used to measure potential; Ag-AgCl electrodes were used for current sending.

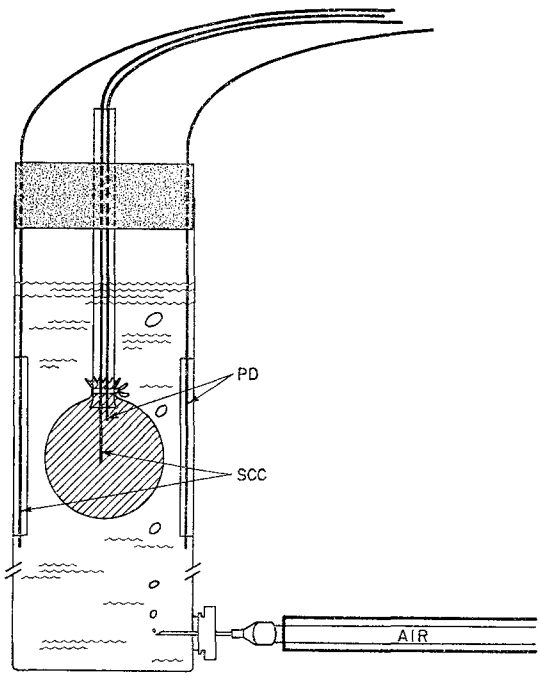

Fig. 1. Sac preparation and vessel used in measurements of transmural potential difference (PD) and short-circuit current (SCC). Bladder is mounted on glass cannula. PD; KCl-agar bridges leading to potential-measuring electrodes. SCC; KCl-agar bridges leading to current-sending electrodes. Outer bridges are threaded through glass channels fused to sides of vessel

Fig. 1 shows the vessel used in electrical experiments. The vessel was a glass cylinder with a rubber-capped opening at the lower edge through which a hypodermic needle was inserted for aeration. Volumes inside and outside of the sac were 10 ml and 60 ml, respectively. Fine polyethylene bridges containing 1% agar in saturated KCl extended from sacs to test tubes containing saturated KCl in which the electrodes were immersed. Electrode potentials between the ends of the PD-measuring bridges were tested frequently and usually found to be negligible. The arrangement of bridges is shown in Fig. 1. The bridges were withdrawn from the sacs after each measurement to prevent leaching of KCl during the experiments. KCl in bathing fluids was measured at the beginning and end of some experiments and found to remain constant. To avoid the effects of stretching on PD and SCC [32], no changes in sac contents were made after the bladders were set up. After preparation, bladder sacs were allowed to equilibrate for 30 to 60 min after which recording of SCC and PD was begun. Experiments were conducted at room temperature, approximately 22 °C.

The composition of the depletion Ringer's in terms of final concentrations was NaCl, 103 mM; $NaHCO_3$, 3 mM; KCl, 3 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM. For substrate-containing Ringer's solution, NaCl was reduced and glucose, 5.5 mM, and sodium pyruvate, 2 mM, were added. Osmolality of both substrate-containing and substrate-free Ringer's solutions was adjusted to 224 mosm/kg and pH to 7.8. Streptomycin sulfate, 5 mg%, was added to all solutions to depress bacterial contamination.

Results

Bacterial Contamination

Bacterial assays were made of substrate-free, streptomycin-containing Ringer's solutions in 6 experiments before and after incubation of bladders. Plate counts revealed 300 to 1,600 bacteria per ml in initial bathing solutions. The number approximately doubled during the 18-hr incubation at room temperature. The bacteria were predominantly Gram-negative rods. Using, as a first approximation, Hershey and Bronfenbrenner's value for the maximum rate of oxygen consumption of *E. coli* in beef broth at 37 °C, 11×10^{-7} mm^3/hr per organism [12], the total oxygen consumption of 1,000 bacteria per ml in the 10-ml respirometer vessel would be 8.3×10^{-6} μmoles per min. Other values for bacterial respiration are smaller [7, 12]. The rates of oxygen disappearance measured in the respirometer during experiments with bladder tissues ranged from 0.02 to 0.11 μmole per min. Since the error using the high value for bacterial oxygen consumption and low value for tissue respiration is less than 0.05%, the contribution of bacterial respiration to total oxygen consumption must have been negligible even at low tissue respiration rates.

Effects of Depletion on Whole Bladder Tissue

Table 1 shows oxygen consumption, glycogen levels, and electrical parameters of paired fresh and depleted hemibladders. Oxygen consumption showed the smallest decline; the respiration rate after 18 hr in substrate-free Ringer's was approximately 61% of original. Glycogen content fell considerably, but the depleted level, 36% of original, was still appreciable. PD

Table 1. Effects of incubation in substrate-free Ringer's solution on turtle bladder

Parameter	N	Initial level	Depleted level	*P*
O_2 consumption (μmoles/min per g dry wt)*	6	1.62 ± 0.08	0.99 ± 0.08	<0.001
Glycogen (mg/g wet wt)	12	2.72 ± 0.20	0.97 ± 0.09	<0.001
PD (mV)*	17	74.1 ± 8.3	20.8 ± 4.5	<0.001
SCC (μamps)*	17	326.1 ± 29.4	60.5 ± 16.2	<0.001

* Values are means for 1 hr of observation. All values are means ± SEM. N, number of experiments. Incubation time, 18 hr. Initial values were obtained after an equilibration period of 30 to 45 min. The experiments were performed from July through September, 1970.

and SCC were highly variable with respect to both initial levels and behavior during incubation. PD usually was well-maintained during the first 4 to 5 hr of incubation, then fell to low levels overnight. However, an occasional bladder showed good maintenance or even increases in PD after 18 hr in substrate-free Ringer's solution. SCC showed the greatest decline of the parameters in Table 1, falling to 19% of original after 18 hr of depletion.

Effects of Depletion on Glycogen Content and Oxygen Consumption of Mucosal and Serosal Fractions of Turtle Bladder

To analyze the depletion process in more detail, the oxygen consumption and glycogen content of dissected and isolated mucosal and serosal fractions of turtle bladder were determined. The two types of analysis were not made on the same bladders, but were carried out during the same weeks using turtles from the same shipment. The findings delineate aspects of the metabolism of two major cell types, epithelial and smooth muscle cells. It was not possible to obtain isolated bladder connective tissue, but experiments with other connective tissues, ligament and subdermal connective tissue, showed both to be essentially glycogen-free and nonrespiring. Thus, the collagenous connective tissue present in both fractions can be assumed to be largely inert with respect to both glycogen content and respiration.

Oxygen Consumption. Fig. 2 shows oxygen consumption levels and response to substrate addition of fresh and depleted bladder fractions. Mean oxygen consumption (μmoles/min per g dry wt) in the period before substrate addition was 3.19 for fresh mucosa and 0.39 for fresh serosal tissue. Mean oxygen consumption before substrate addition was 1.54 for depleted mucosa and 0.37 for depleted serosa. Thus serosal oxygen uptake was little changed by depletion while mucosal respiration declined to less than half of the original rate. To compare other smooth muscle with bladder serosal tissue, the respiration of smooth muscle from the turtle small intestine was investigated using procedures analogous to those used for bladder serosal tissue. Intestinal smooth muscle, after depletion, also respired at approximately the same rate as matching fresh tissue. This finding of long-term constancy of oxygen uptake of smooth muscle, although unexpected, is precedented by observations on skeletal muscle. Smilie and Manery [30] noted that respiratory rates of frog sartorii soaked in substrate-free Ringer's solution for 18 hr were similar to rates reported by others for fresh sartorii.

To determine if the response of respiration to substrate addition was altered by the depletion process, 5.5 mM glucose plus 2 mM Na pyruvate

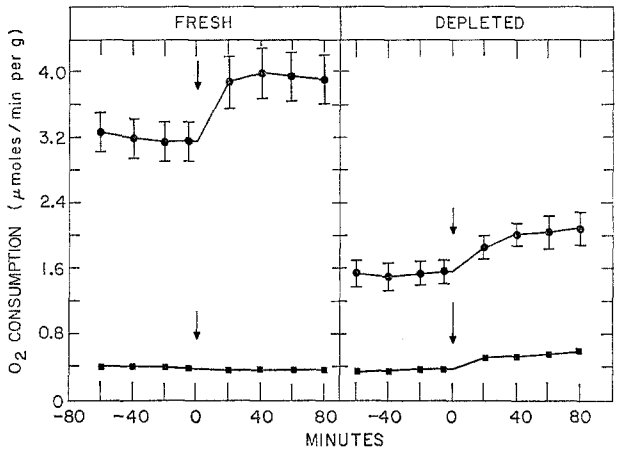

Fig. 2. Effect of depletion procedure and substrate addition on oxygen consumption of mucosal (circles) and serosal (squares) fractions of turtle bladder. At arrows, solution bathing slices of designated tissue was changed from substrate-free Ringer's solution to Ringer's solution containing 5.5 mM glucose plus 2 mM sodium pyruvate. Vertical lines show standard error for mucosal fraction. Standard error for serosal fraction was no more than twice the width of the square marking the point. Number of experiments, 6 for each curve

was added to both fresh and depleted tissues. As shown in Fig. 2, both fresh and depleted mucosal fractions responded to substrate addition. One hr after substrate addition, the oxygen consumption rates of fresh and depleted mucosal tissue were 125 and 131%, respectively, of pre-addition rates. The increases were significant ($P<0.01$ for paired differences in oxygen uptake at -5 and $+60$ min for both fresh and depleted mucosa).

The oxygen uptake of fresh serosal tissue was not significantly altered by substrate addition. In contrast, the respiration of depleted serosa was stimulated; the rate 1 hr after substrate addition was 148% of the pre-addition rate. The increase was significant ($P<0.01$ for paired differences between rates at -5 and $+60$ min). Depleted serosal tissue, after substrate addition, respired at a greater rate than fresh serosal tissue.

Since the serosal fraction makes up 60 to 70% of bladder wet weight [18], the constancy of serosal respiration was responsible for the relatively good maintenance of whole bladder oxygen consumption (Table 1).

Glycogen Content. Table 2 shows the effects of incubation in substrate-free media on glycogen content of isolated mucosal and serosal fractions. Bladders for these experiments were incubated in aerated substrate-free Ringer's solution for 18 hr as described previously. At the end of the incubation period, the bladders were dissected into mucosal and serosal components

Table 2. Effects of depletion on glycogen content of mucosal and serosal fractions of turtle bladder

Fraction	N	Incubation time (hr)	Initial level (mg/g)	Depleted level (mg/g)	Δ	P
Mucosal	6	6	2.28 ± 0.15	1.67 ± 0.23	-0.62 ± 0.18	<0.02
Serosal	6	6	2.82 ± 0.41	1.96 ± 0.36	-0.86 ± 0.29	<0.05
Mucosal	6	18	2.15 ± 0.13	0.76 ± 0.04	-1.39 ± 0.12	<0.001
Serosal	6	18	2.99 ± 0.16	0.94 ± 0.10	-2.05 ± 0.20	<0.001

All values are means $\pm$SEM. N, number of experiments. Glycogen content, mg glycogen (as glucose) per g wet tissue weight. Δ, difference between paired initial and final values. P, significance of difference between paired initial and final values. The experiments were performed in May, 1970.

and the analyses performed immediately. Table 2 shows that lack of substrate did not cause rapid loss of glycogen stores; approximately 70% of the original content was still present in both mucosal and serosal fractions after 6 hr. Considerable glycogen remained even after 18 hr of depletion. The remaining glycogen was 35 and 31% of original in mucosal and serosal fractions, respectively.

Resynthesis of Glycogen

The study of glycogen synthesis *in vitro* in vertebrate liver and skeletal muscle has provided information on metabolic pathways in these tissues [2, 22, 23]. To see if glycogen synthesis takes place in the depleted turtle bladder, glycogen levels were determined before and after the addition of substrate.

Hemibladders were incubated for 18 hr in substrate-free Ringer's solution. At the end of this period, the control hemibladders were dissected into mucosal and serosal fractions and glycogen analyses made. The experimental hemibladders were transferred to Ringer's solution containing 5.5 mM glucose and 2 mM pyruvate. After 5 hr in the enriched solution, the hemibladders were dissected into mucosal and serosal fractions and glycogen analyses were made.

The results of the experiments, shown in Table 3, indicate that substantial net synthesis of glycogen took place in both smooth muscle and epithelial tissue as a result of exposure to substrate. The net increase per gram wet

Table 3. Resynthesis of glycogen by depleted bladders

Fraction	N	Incubation time (hr)	Depleted level (mg/g)	Enriched level (mg/g)	Δ	P
Mucosal	6	5	0.88±0.08	1.59±0.18	0.72±0.22	<0.01
Serosal	6	5	1.01±0.06	1.65±0.11	0.69±0.07	<0.001

All values are means ±SEM. N, number of experiments. Glycogen content, mg glycogen (as glucose) per g wet tissue weight. Δ, difference between paired initial and final values. P, significance of difference between paired initial and final values. The experiments were performed in May, 1970.

tissue weight was nearly the same for both fractions, 0.72 mg for mucosal tissue and 0.69 mg for serosal tissue. Mucosal glycogen increased to 179% and serosal glycogen to 163% of the depleted level.

Effects of Substrate Addition on PD and SCC of Depleted Bladders

Fig. 3 shows the effects of substrate addition on electrical parameters. The bladders used for these experiments were depleted as described previously. After 18 hr of depletion, the bladders were transferred to Ringer's solution enriched with 5.5 mM glucose plus 2 mM pyruvate. The Ringer's

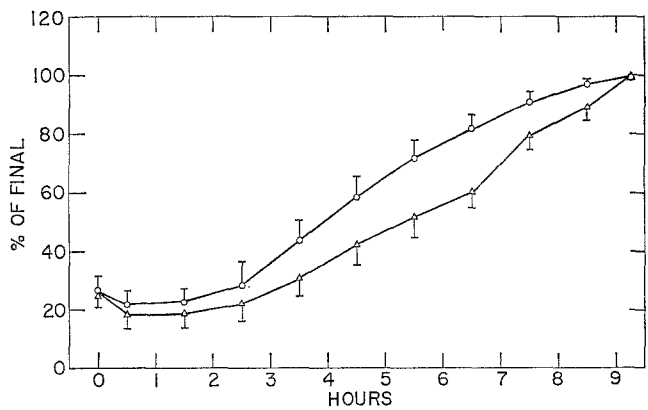

Fig. 3. Effect of substrate addition on transmural potential difference (upper curve) and short-circuit current (lower curve) of depleted hemibladders. At time 0 solution bathing the hemibladders was changed from substrate-free Ringer's solution to Ringer's solution containing 5.5 mM glucose plus 2 mM sodium pyruvate. Values are expressed as percent of value $9\frac{1}{2}$ hr after substrate addition. Vertical lines show one-half standard error. Number of experiments, 9

was aerated and brought to room temperature before the transfer. No change of sac contents was made. Because of the large variability of PD and SCC, the values were expressed as percent of the final value. The final value was taken 9½ hr after substrate addition, this being the approximate time of the maximal increase in PD. SCC continued to increase for several hours longer. Mean increments ±SE for PD and SCC in the 9½-hr period after substrate addition were 24.8±5.0 mV and 98.2±13.3 μamps, respectively; (9 experiments).

As shown in Fig. 3, the immediate response of PD and SCC to the transfer to substrate-enriched Ringer's was a slight decrease followed by a lag period. A similar lag period was seen in experiments in which substrate was added by injecting a small volume of concentrated solution into the outside bathing medium. After about 2 hr both PD and SCC began to increase and continued to increase for many hours. The long-lasting increases in PD and SCC shown in Fig. 3 differ from those described for toad bladder. Edelman, Bogorach and Porter [5] found that addition of glucose or pyruvate to depleted toad bladder caused an immediate stimulation of SCC that was maximal at 1 to 2 hr and thereafter declined unless aldosterone had been added previously.

Water Content of Fresh and Depleted Bladder Fractions

To permit conversion from wet to dry weight in calculations, water content were determined for fresh and depleted mucosal and serosal fractions. The results, shown in Table 4, indicate that the serosal fraction was significantly denser than the mucosal fraction in both fresh and depleted tissues. Mean values for g H_2O per g dry wt ±SE for fresh and depleted mucosal tissue were 6.38±0.12 and 5.69±0.15, respectively, and for serosal tissue were 6.56±0.30 and 6.01±0.30, respectively.

Table 4. Water content of fresh and depleted bladder fractions

Bladder	N	Incubation time (hr)	Mucosal fraction g H_2O/ g dry wt	Serosal fraction g H_2O/ g dry wt	*Δ*	*P*
Fresh	5	0	6.38±0.12	5.69±0.18	0.70±0.15	<0.01
Depleted	5	18	6.56±0.30	6.01±0.30	0.55±0.08	<0.01

All values are means ±SEM. N, number of determinations. *Δ*, difference between paired initial and final values. *P*, significance of difference between paired initial and final values. The determinations were carried out in June, 1970.

Discussion

The present study shows that the depleted turtle bladder is a well-regulated system with considerable capacity for response despite its long removal from the neuronal and hormonal control mechanisms of the intact animal. The depletion procedure, incubation in aerated substrate-free Ringer's solution for 18 hr at room temperature, apparently did not cause severe irreversible losses of enzymes and cofactors necessary for stimulation of the parameters studied (oxygen consumption, glycogen content, SCC and PD).

At the end of depletion, the parameters were not depressed uniformly. Considering the bladder as a whole, oxygen consumption was least affected; the rate of whole bladder respiration after 18 hr was 61% of original. Glycogen levels were 36% of original, while PD and SCC were only 28 and 19%, respectively, of original. Electrical parameters, however, are more properly compared with parameters of the mucosal portion of the bladder since the ion transport processes that generate PD and SCC are believed to take place in epithelial cells. Fig. 2, illustrating the respiration of isolated mucosal and serosal fractions of the bladder, shows that the respiration of the mucosal fraction declined by approximately one-half during the depletion period. Table 2 shows that the glycogen level of depleted mucosal tissue was 35% of original. The four parameters, oxygen consumption, glycogen content, PD and SCC, thus act independently whether considered for the whole bladder or for the mucosal fraction alone. SCC, for example, does not mirror total oxygen consumption. Partial coupling of these parameters is not, of course, ruled out.

Electrical Parameters and Substrate Addition

The behavior of SCC and PD after transfer of bladders to a solution containing glucose and pyruvate (Fig. 3) requires closer examination. The absence of stimulation during the first 2 hr resembles an induction period and is in contrast to the prompt stimulation of oxygen consumption after addition of substrate (Fig. 2). However, since the turtle bladder is believed to transport chloride and bicarbonate ions in addition to sodium ion [9, 27], the behavior of PD and SCC after exposure to substrate could be caused by the ratio of cations to anions transported. If anion and cation transport occurred at equal rates after the admission of substrate, but cation transport gradually increased relative to anion transport, the lag phase (first 2 hr) shown in Fig. 3 would be accounted for. Further experiments will be necessary to determine whether ion transport and oxygen consumption truly have disparate responses to substrate addition.

Response of Serosal Respiration to Substrate Addition

The pattern of response of fresh and depleted serosal tissue to substrate addition is of interest (Fig. 2). The oxygen consumption of fresh serosal tissue showed no response to substrate while that of depleted serosal tissue was clearly stimulated. Resting skeletal muscle is known to be relatively impermeable to glucose [14] and it may be surmised that smooth muscle is also. However, the lack of response of fresh smooth muscle to glucose plus pyruvate is almost certainly not due to the total impermeability of the cells to these substances. It is probably due to the holding down of respiration by regulatory mechanisms [8, 13] of which low permeability to substrate is only one. Depleted smooth muscle, in contrast, probably has lost ATP and respiration-inhibiting intermediates and possesses increased permeability to substrates as well. The effectiveness of compartmentalization of enzymes may have decreased also.

Comparison of Oxygen Consumed with Glycogen Utilized

Table 5 gives an estimate of the amounts of glycogen (as glucose residues) and oxygen consumed by mucosal and serosal tissue during the 18 hr of depletion. These values provide information on the endogenous substrates utilized by epithelial and smooth muscle cells. Since the complete combustion of a mole of glucose utilizes 6 moles of oxygen, ratios of oxygen consumed to glucose disappearing in excess of 6 indicate that substrates in addition to glucose have been oxidized.

Moles of glucose consumed were calculated for mucosal and serosal fractions by subtracting the glycogen (as glucose) content of depleted tissue from that of fresh tissue (Table 2). Wet/dry ratios derived from Table 4 were used to convert wet weight to dry weight. Amount of oxygen consumed during the depletion period can be calculated from the respiration rate if this is known during the entire period. It could be calculated with reasonable certainty for the serosal fraction where the oxygen uptake rate was almost constant. However, the mucosal fraction did not respire at a constant rate; thus a definite value for moles of oxygen consumed cannot be given. But, making the likely assumption that the rate during the depletion period stayed within the range bounded by initial and final rates, a range of values for total oxygen consumption was found. From this range, a range limiting the possible values of the ratio of oxygen consumed to glucose utilized was calculated for the mucosal fraction. The oxygen consumed by the serosal fraction was handled similarly although the range was small. The ranges of oxygen/glucose consumed, 30 to 61 for the mucosal fraction and 5.4 to 5.6

Table 5. Comparison of O_2 consumed with glycogen utilized in mucosal and serosal fractions of turtle bladder

	N	Mucosal fraction		Serosal fraction	
		Fresh	Depleted	Fresh	Depleted
O_2 Consumption (μmoles · min^{-1} · g^{-1} dry wt)	6	3.19	1.54	0.39	0.37
Glycogen content (μmoles glucose/g dry wt)	6	88.1	31.9	111.0	36.6
Total O_2 consumption (range) (μmoles/g dry wt)		3445.2–1663.2		421.2–399.6	
Total glucose utilized (μmoles/g dry wt)		56.2		74.4	
Limits of ratio (O_2/glucose)		61.3–29.6		5.6–5.4	

Depletion time, 18 hr. N, number of experiments.

for the serosal fraction, indicate that glycogen does not make up the same proportion of endogenous fuel in the two tissues. The entire mucosal range is incompatible with the utilization of glycogen as the major substrate for oxidation and suggests that lipid is being utilized in addition to glycogen. The levels of lipids in turtle bladder epithelial cells have not been investigated, but it is of interest that toad bladder epithelial cells contain large stores of triglycerides which appear, because of their rapid turnover, to be a major substrate [26]. The data of Table 5 strongly suggest that the turtle bladder mucosa also utilizes lipid preferentially under aerobic conditions. The range of values bounding the serosal ratio, on the other hand, is compatible with the utilization of glycogen as the major substrate although it does not prove that this is the case. Because of the uncertainty of applying measurements of oxygen consumption of slices to sacs (*see* Materials and Methods) and because lactate production and gluconeogenesis were not measured, the calculations of Table 5 must be regarded as approximations. The difference in the ratios of oxygen consumed to glycogen utilized for epithelial and smooth muscle tissue, however, is great enough to be a clear indication of the existence of very different types of energy metabolism in these tissues.

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