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EVIDENCE FOR A SODIUM ION EXCHANGE CARRIER LINKED WITH GLUCOSE TRANSPORT ACROSS THE BRUSH BORDER OF A FLATWORM (*HYMENOLEPIS DIMINUTA*, CESTODA)

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SUMMARY

The manner in which the flatworm, *Hymenolepis diminuta* (Cestoda), regulates the transport of glucose and Na^+ across the brush border was examined. While the presence of an unstirred region in the brush border may favor the reabsorption of leaked glucose, some leaked glucose was lost to the ambient medium. This loss was markedly enhanced by preloading the worms with glucose and by removing Na^+ from the incubation medium. Since glucose and Na^+ influxes are coupled, glucose leakage stimulated the influx of $^{22}Na^+$. However, this $^{22}Na^+$ influx was balanced by a simultaneous increased $^{22}Na^+$ efflux. The presence of phlorizin inhibited both unidirectional fluxes of $^{22}Na^+$ indicating that efflux of $^{22}Na^+$ occurred by countertransport; countertransport of [^{14}C]glucose appeared to be negligible. A model has been proposed in which the transport of glucose and compensating transfers of Na^+ across the membrane occur via the same carrier.

INTRODUCTION

Most parasitic flatworms can absorb nutrients through the tegument. Transport across the brush border is considered to be the first step in this process, and in those flatworms which lack a gut, its importance is undeniable. On the basis of in vitro kinetic studies describing the unequal distribution of solutes between the cytoplasm and the ambient medium, the presence of specific transport systems in the plasma membrane have been postulated.

The absorption kinetics of glucose has been extensively studied in the tapeworm, *H. diminuta*. The transport process exhibits a first order dependence on the concentration of Na^+ in the external medium [1] and coefficients of coupling of glucose and Na^+ have been estimated [2]. It has also been demonstrated [2] that a high concentration (100 mM) of K^+ does not inhibit the Na^+ activation of glucose transport even when the Na^+ concentration is low (25 mM).

Phlorizin competitively inhibits glucose transport in *H. diminuta* [2, 3] having an affinity for the transport system some 250-times greater than that of glucose. In spite of its inhibitory potency the effects of phlorizin are rapidly reversed by washing

[4]. Since the amount of ^{14}C-labelled phlorizin absorbed by the worm is negligible [5], the site of phlorizin inhibition is probably located at the external surface of the worm.

Read et al. [2] demonstrated by double-labelling experiments that the influxes of glucose and Na^+ in *H. diminuta* are apparently coupled. They also postulated the existence of a separate Na^+ transport system based on the observation that a mediated influx of Na^+ occurred in "glucose-free" media. However, this curious observation is equivocal since some glucose leakage from the worm may have been involved.

Since there is little information available on this latter subject, a more rigorous examination of glucose and Na^+ fluxes in *H. diminuta* was initiated. The effect of various sugars on the efflux rate of [^{14}C]glucose was determined. Glucose efflux was also determined chemically and an attempt was made to correlate the rate of glucose leakage with the simultaneous unidirectional fluxes of Na^+ ($^{22}Na^+$). The effects of phlorizin, ouabain, and various ions on $^{22}Na^+$ influx were also examined. Finally, tissue Na^+, K^+, glucose, and water were measured by direct analysis to determine whether the observed fluxes could be explained in terms of membrane phenomena alone.

MATERIALS AND METHODS

Animals

Infective larvae (cysticercoids) of *H. diminuta* were grown in laboratory stocks of beetles, *Tenebrio molitor*. Young male rats (Holtzman Co.) each were force-fed 30 cysticercoids using a stomach tube. Rats were maintained on Purina Laboratory Chow and water ad libitum. At 11 days postinfection the worms were removed from the intestine of rat for use in the experiments. At this age each worm weighed approx. 60–80 mg wet wt. 11-day-old worms were used in all experiments.

Salines

The salines used were Krebs-Ringer with 25 mM tris(hydroxymethyl)-aminomethane-maleate buffer at pH 7.4 and, where indicated, sodium-free saline in which the NaCl was replaced isosmotically with tris(hydroxymethyl)-aminomethane-chloride, LiCl, KCl, or choline-chloride.

Sodium influx procedure

After removal from the rat the worms were washed and randomized into groups (4 worms/group). Each group was preincubated at 37 °C for 15 min in 10 ml of fresh saline, and then incubated at 37 °C for 2 min in 5 ml of the appropriate saline containing 10 mM ^{22}NaCl (Amersham/Searle). To terminate the incubation the worms were rinsed briefly, blotted on hard filter paper, and extracted in 3 ml of 70 % ethanol for 24 h. Radioactivity in the ethanol extracts was determined using a liquid scintillation spectrometer (Beckman). The worms were dried at 95 °C for 24 h and weighed to the nearest 0.1 mg. Washing, preincubation, and incubation media were of the same ionic composition.

Determination of glucose leakage

Worms (4 worms/assay) were incubated for 1 h in 10 ml of saline containing 5 mM glucose. This procedure raised the tissue glucose, [G_i], of the worm. The worms were removed, rinsed in three changes of prewarmed saline, and then incubated in 2 ml of saline or Na^+-free saline (using KCl to replace the deleted NaCl). After 30 s, 2, 5, 15, 30, and 60 min the worms were removed and dried. Glucose in 0.5 ml aliquots of the incubation media was determined using the glucose oxidase method (Glucostat Special; Worthington Biochemicals).

Countertransport of glucose

By definition glucose influx in *H. diminuta* occurs by active transport. The K_t for glucose transport is approximately 1 mM and at glucose concentrations greater than 5 mM, the system operates at maximal velocity. In an attempt to determine whether the system countertransports glucose, worms were "preloaded" in saline containing 5 mM [^{14}C]glucose for 30 min. The worms were removed, rinsed rapidly three times in prewarmed saline, and then incubated in saline containing unlabelled glucose or 2-deoxyglucose. The latter sugar was included as a control because it does not interact with the glucose transport system. After 2 and 5 min incubations the worms were rinsed, blotted, and extracted in ethanol. Radioactivity in the ethanol extracts was determined as above. Radiochromatographic analyses of the ethanol extracts showed that more than 95 % of the radioactivity was present as glucose.

Glucose and sodium accumulation

In the accumulation studies worms were preincubated in saline for 15 min and then incubated in 10 ml of saline containing 5 mM glucose with or without 1 mM ouabain. Incubations without glucose or ouabain served as controls. After intervals of time up to 1 h the worms were removed, rinsed, and extracted in 70 % ethanol for 24 h. Glucose in 0.5 ml aliquots of the ethanol extracts was determined using the glucose oxidase method. Na^+ and K^+ concentrations in the ethanol extracts were determined using a Baird Atomic flame photometer, Model KY-2C. Standard solutions of Na^+, K^+ and Li^+ in the Cl form were obtained from Baird Atomic, Inc.

Unidirectional fluxes of Na^+

The methods for measuring the unidirectional fluxes of Na^+ are summarized in Table II. Some worms (Groups 3 and 4) were incubated for 1 h in saline containing $^{22}Na^+$, one group with 5 mM glucose to increase the $[G_i]$ and, thus, the rate of glucose leakage. The worms were rinsed in prewarmed saline, blotted dry on hard filter paper, weighed (±0.5 mg), then incubated in 5 ml of fresh saline containing 10 mM Na^+ (without the $^{22}Na^+$ label). Tris · Cl was used to replace the deleted NaCl. After intervals of up to 30 min the worms were rinsed, blotted, re-weighed, and extracted in 70 % ethanol. Based on the radioactivity remaining in the worms, the efflux rates of $^{22}Na^+$ were determined.

Other incubations (Table II; groups 1 and 2) were identical to those described above except that the $^{22}Na^+$ label was present in the second incubation rather than in the first. In this way both the unidirectional fluxes (influx and efflux) of $^{22}Na^+$ could be measured simultaneously. A low Na^+ concentration (10 mM) was chosen for the second incubations to minimize the passive movements of the label. Since significant changes in worm water and $[G_i]$ might occur during incubation, these latter parameters were also determined (Fig. 3; lower graph). The values for $[G_i]$ were calculated on the basis of worm water at the end of each respective incubation period. The data were compared using the Students *t* test.

RESULTS

Sodium influx in 2 min incubations

Unlabelled Na^+ (120 mM) inhibited the influx of 10 mM $^{22}Na^+$ (Table I) indicating the presence of a mediated transport system for Na^+ when glucose is not present in the incubation medium. However, phlorizin also inhibited $^{22}Na^+$ influx. Furthermore, the incubation produced by 120 mM Na^+ plus phlorizin was not significantly greater ($P = 0.05$) than that produced by 120 mM Na^+ alone. Since phlorizin is a potent inhibitor of glucose transport in *H. diminuta*, the apparent mediated influx of $^{22}Na^+$ in "glucose-free" media can be accounted for by a leak-pump involving glucose leakage and subsequent coupled influx with Na^+.

TABLE I

THE EFFECTS OF 1 mM PHLORIZIN AND OUABAIN AND VARIOUS IONS (120 mM) ON THE UPTAKE OF 10 mM $^{22}Na^+$ BY *H. DIMINUTA* IN 2 MIN INCUBATIONS

Tris · Cl was used to replace the deleted NaCl in the controls and incubations with phlorizin and ouabain. The rates are reported as μmol of $^{22}Na^+$ absorbed/g ethanol extracted dry wt./h. Each value is the mean ± S.E. of 5 replicates.

Inhibitor	Rate	Stimulation (S) or inhibition (I) (%)
Control	14.79 ± 0.77	–
Li^+	10.32 ± 0.42	27 I
K^+	13.26 ± 0.60	0
Na^+	7.60 ± 0.66	46 I
Choline	19.39 ± 0.42	38 S
Phlorizin	9.02 ± 0.50	36 I
Ouabain	14.62 ± 0.91	0
Na^+ + phlorizin	7.21 ± 0.74	51 I

Accumulation of glucose and Na^+

During a 1 h incubation in saline plus 5 mM glucose, tissue glucose, $[G_i]$, increased from 5 mM to about 25 mM (Fig. 1). Tissue Na^+, $[Na^+_i]$, also increased while K^+, $[K^+_i]$, did not change. The presence of 1 mM ouabain had no effect on the capacity of the worms to accumulate glucose or Na^+ (not shown). While there was a 2-fold increase in $[G_i]$ in the controls, it has been demonstrated previously [3] that there is a corresponding decrease in glycogen during a 1 h incubation in saline alone. Thus, the increased $[G_i]$ is probably the result of glycogenolysis; not only do worms degrade glycogen in the absence of external glucose, but glucose is the only glycogenic monosaccharide in *H. diminuta* [6].

Glucose leakage

The data in Fig. 2 show the effects of $[G_i]$ and sodium on glucose leakage. When worms were first incubated for 1 h in saline with 5 mM glucose and then transferred to glucose-free media, glucose rapidly appeared in the incubation medium (Fig. 2; middle curve) and continued to increase for 15 min. During the last 45 min of incubation, glucose in the ambient medium gradually decreased. A precipitous in-

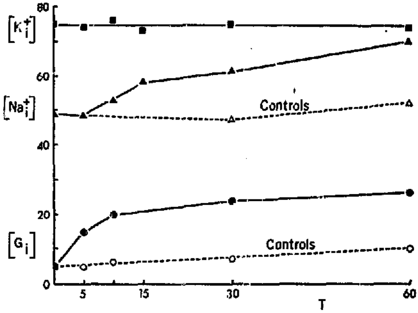

Fig. 1. Tissue Na^+ ($[Na_i^+]$, triangles) and potassium ($[K_i^+]$, squares) during the accumulation of glucose ($[G_i]$, circles) in *H. diminuta*. Closed symbols, worms incubated in saline +5 mM glucose. Open symbols (controls), worms incubated in saline alone. All values are expressed as μmol/ml worm water and are the average of 3 replicates. Since there were no changes in the $[K_i^+]$, only one line is given for clarity. Na^+ and K^+ concentrations were determined by direct analysis using a flame photometer. Glucose was determined using the glucose oxidase method. The presence of ouabain in the incubation medium did not affect the capacity of the worms to accumulate glucose and Na^+ (not shown). *T*, time in minutes.

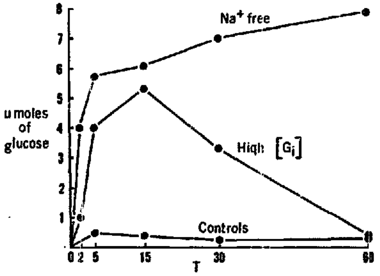

Fig. 2. Chemical determination of glucose leakage in *H. diminuta*. Each assay consisted of 4 worms (approximately 50 mg dry wt.) in 2 ml of incubation medium. In the controls (lower curve) the incubation medium consisted of saline. To increase the glucose leakage, some worms were preincubated for 1 h with glucose, then washed prior to incubation in saline (middle curve). The upper curve represents glucose efflux in Na^+-free saline where the NaCl was replaced isosmotically with KCl. Each point is the average of 3 replicates and represents the total μmol of glucose in the medium/g dry wt. *T*, time in minutes.

crease in medium glucose was also produced by incubation in Na$^+$-free saline (Fig. 2; upper curve). However, glucose accumulated in the medium for the entrie 60 min incubation period.

Effect of glucose and 2-deoxyglucose on [^{14}C]glucose efflux

There was a 10% decrease in tissue [^{14}C]glucose during a 5 min incubation in saline (Table III). When unlabelled glucose (1 or 10 mM) was added to the efflux medium, the loss of [^{14}C]glucose was no greater than that in saline alone. Since the rate of glucose transport did not affect the rate of [^{14}C]glucose efflux, it appears that exchange of glucose between tissue and medium by countertransport is negligible.

Effects of glucose leakage on the unidirectional Na$^+$ fluxes

The methods for measuring the effects of the glucose leak (i.e., efflux by diffusion) on the influx and efflux of ^{22}Na$^+$ are summarized in Table II. ^{22}Na$^+$ influx in

TABLE II

A SUMMARY OF THE METHODS USED TO DETERMINE THE EFFECT OF GLUCOSE LEAKAGE ON THE UNIDIRECTIONAL FLUXES OF ^{22}Na$^+$ IN *H. DIMINUTA*

Group	Preincubation medium (1 h)	Rinse, weigh	Incubate for up to 30 min	Rinse, weigh and extract in ethanol
1	Saline (control)	→	10 mM ^{22}Na$^+$	→ μmol ^{22}Na$^+$ in worm determined
2	Saline+glucose		(Na$^+$ influx)	
3	^{22}Na$^+$	→	10 mM unlabelled Na$^+$	
4	^{22}Na$^+$+glucose		(Na$^+$ efflux)	

TABLE III

EFFECT OF GLUCOSE ON [^{14}C]-GLUCOSE EFFLUX FROM *H. DIMINUTA* (PRELOADED IN MEDIA CONTAINING 5 mM [^{14}C]GLUCOSE FOR 30 MIN)

2-Deoxyglucose was also tested as a control since it does not interact with the glucose transport system. The values represent μmol of [^{14}C]glucose remaining in the worms/g ethanol extracted dry wt. Each value is the mean ±S.E. of 3 replicates. [^{14}C]-glucose efflux rates in the presence of glucose and 2-deoxyglucose were not significantly different from the saline control ($P \geq 0.05$).

Sugar	Efflux time (min)	
	2	5
None (saline control)	46.9±1.39	41.6±0.86
Glucose		
1 mM	47.2±2.32	41.9±1.35
10 mM	51.9±4.38	40.6±0.81
2-Deoxyglucose		
1 mM	47.4±2.96	42.8±2.82
10 mM	44.5±2.80	42.0±1.11

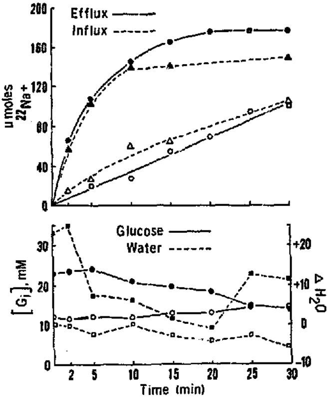

Fig. 3. Effects of a high $[G_i]$ on the unidirectional fluxes of $^{22}Na^+$ in *H. diminuta* (see text and Table II for details). Worms (group 2) were equilibrated for 1 h in saline containing 5 mM glucose. The influx of 10 mM $^{22}Na^+$ was then determined (upper graph; solid triangles). Other worms (group 4) were equilibrated in saline containing 5 mM glucose and $^{22}Na^+$. The efflux of $^{22}Na^+$ from these worms in saline containg 10 mM unlabelled Na was determined (upper graph; solid circles). Worms equilibrated in saline lacking glucose (groups 1 and 3) served as controls (upper graph; open triangles and open circles, respectively). Tris · Cl was used to replace the deleted NaCl. The values are expressed as μmol $^{22}Na^+$/g ethanol extracted dry wt. The absolute rates of influx and efflux for 2, 5, and 10 min are given in Table IV. At each time period the $[G_i]$ and Δ worm water were also determined (lower graph; circles and squares, respectively; solid symbols, experimentals; open symbols, controls). Each assay was conducted in replicates of 3.

TABLE IV

EFFECTS OF GLUCOSE LEAKAGE ON THE UNIDIRECTIONAL FLUXES OF $^{22}Na^+$ IN *H. DIMINUTA*

See text and Fig. 3 for details. The influx rates are expressed as μmol of $^{22}Na^+$ absorbed (±S.E.)/g ethanol extracted dry wt./2,5, and 10 min. Efflux rates represent μmol $^{22}Na^+$ lost (±S.E.)/g ethanol extracted dry wt./2,5, and 10 min. Each assay was conducted in replicates of 3. The absolute values for influx and efflux at each time period were not significantly different ($P > 0.05$).

Incubation time (min)	Influx	Efflux
2	66.24±0.24	68.01±0.85
5	105.86±1.58	109.28±3.83
10	137.39±1.61	143.50±7.81

the controls (Fig. 3; open triangles) was approximately linear up to 30 min, while $^{22}Na^+$ influx in worms preloaded with glucose (Fig. 3; closed triangles) was markedly stimulated for 10 min. This stimulatory effect corresponded with the enhanced glucose leak represented in Fig. 2. $^{22}Na^+$ influx then sharply decreased at about 10 min.

The rate of glucose leakage also affected the rate at which $^{22}Na^+$ left the worms. Efflux from controls (Fig. 3; open circles) was a linear function of time while efflux from the experimentals was stimulated (Fig. 3; closed circles). Thus, an elevated $[G_i]$ enhanced both unidirectional fluxes of Na^+. A comparison of the data (Table IV) revealed that the magnitude of the unidirectional $^{22}Na^+$ fluxes was the same up to 10 min. After 10 min there was a net loss of the label.

Effects of phlorizin on Na^+ efflux

It is recalled that phlorizin inhibited $^{22}Na^+$ influx (Table I). Phlorizin also inhibited $^{22}Na^+$ efflux (Fig. 4) indicating that both directional movements of sodium occurred via the same "carrier".

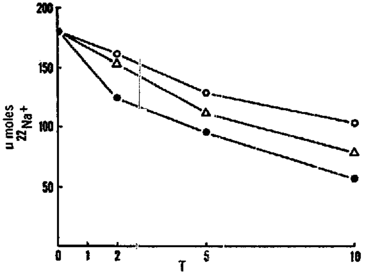

Fig. 4. Effect of phlorizin on the efflux of $^{22}Na^+$ from *H. diminuta*. Worms were equilibrated in saline containing 5 mM glucose and $^{22}Na^+$ for 1 h. Efflux of $^{22}Na^+$ into 3.5 ml of saline containing 10 mM unlabelled Na^+ (middle curve, open triangles), 10 mM unlabelled Na^+ plus 0.5 mM phlorizin (upper curve, open circles), and 154 mM Na^+ (lower curve, closed circles) was determined. Tris · Cl was used to replace NaCl. Each assay was conducted in replicates of 5. The values were determined from the radioactivity remaining in the worms and represent μmol $^{22}Na^+$/g ethanol extracted dry wt.

DISCUSSION

The data presented in Table III show that tissue glucose of *H. diminuta* does not readily exchange with medium glucose by countertransport, i.e., the presence of 10 mM glucose in the efflux medium did not enhance the rate of [^{14}C]glucose efflux from the worms. This observation suggests that glucose efflux occurs mainly by diffusion, a process which can be termed a glucose leak.

As was expected the rate of glucose leakage (determined chemically) was enhanced by increasing the $[G_i]$ of the worms. However, there was a transition from leakage to absorption at about 10 min followed by a gradual decrease in medium glucose to the level of the controls. A glucose leak was also evident when Na^+-free saline was used as the bathing medium. Previous studies [7, 8] suggest that glucose leakage is a natural process in tapeworms.

With this in mind, it has been demonstrated [2] that *H. diminuta* transports Na^+ by some mediated system in "glucose-free" media. However, demonstration that worms leak glucose suggests that this Na^+ transport is, in fact, influx coupled with glucose leaked from the worm. This conclusion is independently supported by the fact that phlorizin, a competitive inhibitor of glucose transport, abolished this Na^+ transport activity. In this respect the movement of glucose across the brush border of *H. diminuta* resembles a leak-pump.

The data in Fig. 3 not only give support to the direct association between glucose leakage and Na^+ fluxes, but also show the close correspondence between the rates of inward and outward Na^+ movements; the influx and efflux rates of $^{22}Na^+$ were the same in incubations up to 10 min. The fact that phlorizin inhibited influx as well as efflux of $^{22}Na^+$ suggests further that both unidirectional fluxes of Na^+ occur via the same system. If this concept is accurate, then *H. diminuta* can maintain electroneutrality during the initial step in glucose transport by an exchange of Na^+ across the plasma membrane rather than by co-transport of an anion. It is reasonable that such a mechanism would favor the conservation of energy and the maintenance of homeostasis in that region of the worm involved in transport. Clearly, present techniques for determining coupling coefficients of glucose and Na^+ in such systems based on the net amount of $^{22}Na^+$ absorbed in "rapid transfer" incubations must be re-evaluated.

It should be noted that water loss alone might cause the loss of some cellular solutes. Although there was some shrinkage of the worms during the first 10 min of incubation (Fig. 3; lower graph), it was insufficient to account for the observed Na^+ efflux during this time. Furthermore, if a major portion of the observed Na^+ efflux originated from the shrinkage phenomenon, one would also expect a decrease in tissue glucose; no such change in $[G_i]$ was observed. It is also noteworthy that the incubation media contained low (10 mM) sodium. Thus, *H. diminuta* is able to maintain (for at least 25 min) a high $[G_i]$ in a medium in which a reversed Na^+ gradient from normal is imposed.

It seems relevant at this point to compare glucose transport in *H. diminuta* with that in the mammalian intestine. The tapeworm surface consists of a plasma membrane which is maximized by the presence of microvilli. The cytoplasmic layer beneath the plasma membrane is a syncytium. While fluid movement or solvent drag through tight junctions is an important pathway for solute transport in the mammalian intestine [9], there are no tight junctions or other extracellular pathways in tapeworms. Thus, since tapeworms lack a gut of any kind, the absorption of water and solutes must occur through the plasma membrane alone.

While there is a net transcellular movement of Na^+ during the active transport of glucose in mammalian intestine, Cl^- movement in the same direction is sufficient to maintain electroneutrality [10]. On the other hand, the rate of glucose transport and accumulation in *H. diminuta* is unchanged in Cl^--free media in which other anions are present as isosmotic replacements for the deleted Cl^- [11]. Although some inhibition of glucose transport was observed when CH_3OO^- was used as the replacement ion, there was no inhibition when NO_3^- was used. The failure to find evidence that Cl$^-$ is directly involved in glucose transport is consistent with the data of the present paper.

Model of the glucose transport system in H. diminuta

An adequate model of the glucose transport system in *H. diminuta* must account for all of the properties of the system described above. These are: (1) influxes of glucose and Na$^+$ are coupled. (2) Efflux of glucose by countertransport is negligible. (3) Na$^+$-coupled glucose influx stimulates Na$^+$ efflux and the ratio of Na$^+$ influx to Na$^+$ efflux is 1 : 1. (4) Phlorizin inhibition of Na$^+$-coupled influx decreases the rate of Na$^+$ efflux indicating that Na$^+$ efflux occurs by countertransport, i.e., by the same system. The model must also explain the observation that glucose influx does enhance the net uptake of labelled Na$^+$ from the ambient medium indicating that both Na$^+$ and glucose dissociate from the "carrier" upon reaching the inside of the cell.

The current view of membrane structure in which the membrane is comprised of protein "icebergs" floating in a "sea" of lipid was described by Singer [12]. In this hypothesis the transport proteins or "carriers" extend all the way through the membrane. Water-filled channels across the membrane may be formed by groupings of four (or more) protein subunits. A conformational change in the spatial relationship of the subunits (with or without the assistance of chemical energy) would, in effect, "force" a molecule through the channel and eject it on the other side.

Fig. 5 represents a two-dimensional model that accounts for the fluxes of glucose and Na$^+$ in *H. diminuta*. The model is comprised of the substrate glucose, Na$^+$, and an asymmetric carrier. The carrier is visualized as consisting of at least six subunits of protein forming two channels across the membrane. Na$^+$ and glucose molecules (outside) impinge on the active center of the influx channel. Simultaneously, Na$^+$ (inside) is bound to the active center of the efflux channel. The carrier is asymmetric in that the active centers of the channels are structurally separate, and that

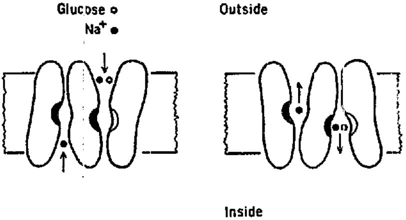

Fig. 5. A two-dimensional model of the glucose transport system in *H. diminuta*. The carrier consists of six subunits of protein forming two structurally separate channels across the membrane. The darkened and outlined areas are the active centers of the channels. The carrier is asymmetric in that glucose, (○) and Na$^+$, (●) from the outside are attracted to the active center of one channel, while Na$^+$ alone is attracted to the other. The conformational change in the carrier which transports glucose and Na$^+$ into the cell through one channel is accompanied by a complementary conformational change which ejects Na$^+$ from the cell through the other.

binding of Na$^+$ at the active center of the efflux channel does not require glucose. The conformational change in the carrier that "forces" glucose and Na$^+$ through one channel is accompanied by a complementary conformational change that ejects Na$^+$ from the cell through the other. Because of its asymmetry operation of the system would result in the net accumulation of glucose inside the cell. At the present time no easy method of experimentally testing how chemical energy may be used by the system is available.

The model described above also predicts that operation of the carrier requires the presence of Na$^+$ on both sides of the membrane. Thus, inhibition of Na$^+$ binding inside the cell would inhibit the coupled influx of glucose and Na$^+$. Another prediction of the model is that operation of the system is nonelectrogenic in that charge compensating transfers of Na$^+$ across the membrane maintain electroneutrality. Both of these predictions are amenable to experimental examination which might lend further support to the model.

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