71-26,361

SOUTHWORTH, George Clark, 1944-
CARBOHYDRATE TRANSPORT IN TRYPANOSOMA
GAMBIENSE.

Rice University, Ph.D., 1971
Biochemistry

University Microfilms, A XEROX Company, Ann Arbor, Michigan

RICE UNIVERSITY

CARBOHYDRATE TRANSPORT IN TRYPANOSOMA GAMBIENSE

by

^{GEORGE}
G. CLARK SOUTHWORTH

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Thesis Director's signature:

A handwritten signature in cursive script, reading "Clark P. Read". The signature is written in dark ink and is positioned above a horizontal line.

Houston, Texas

May 1971

ACKNOWLEDGEMENTS

I wish to thank John Richard Seed for originally teaching me the pleasure of biological research; his friendship and confidence were the foundation of my career. I am also grateful to Clark P. Read whose kind support, subtle instruction and intellectual stimulation will not be forgotten.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
INTRODUCTION	1
A. General Considerations	1
B. Concept of the Carrier and Transport	2
C. Transport and Its Relation to Metabolism	6
D. The Present Study	8
MATERIALS & METHODS	10
A. The Organism	10
B. Host Animals	11
C. Anesthesia	11
D. The Infection	11
E. Maintenance of Parasites	12
F. Infections	13
G. Collection & Preparation of Trypanosomes	13
H. Limitations of Experimental Design	14
I. Incubations	15
J. Determination of Radioactivity	15
K. Reactants & Substrates	15
RESULTS	17
A. The Significance of Isotopic Extraction	17
B. The Absorption of Glucose	17
C. Variation and Reproducibility	19
D. Effect of Temperature	21
E. The Absorption of Other Substrates	21
F. Competition Among Substrates	23
G. Specificity of Glucose Absorption	31
H. Effect of Metabolic Poisons and Other Inhibitors	34
I. Effect of Cation Replacement	37

DISCUSSION	39
A. Glucose Absorption and Relation to Metabolism	39
B. The Effect of Temperature	48
C. Absorption of Carbohydrates Other than Glucose	49
D. Competition and Inhibition	50
E. Specificity of the Glucose Site	69
F. Effect of Metabolic Poisons and Other Inhibitors	73
G. Effect of Cation Replacement	75
APPENDIX A	80
BIBLIOGRAPHY	81
FIGURES (1 - 27)	

INTRODUCTION

A. General Considerations. The interaction of any organism with its environment is a fundamental quality of life. When one organism provides a part or all of the environment for another, a special relationship exists, and the interaction of a parasite with its host is such a relationship. To a parasite, nutritional factors provided by the host environment are of paramount importance, and as a consequence, parasitism is often defined on a nutritional basis. However, the requirement for metabolizable substrates from the environment is not unique to parasites but is of vital importance to all organisms. It follows that the means of acquiring nutrients is of equal importance, and while the requirement for food substances is invariant, the mechanisms of nutrient acquisition are as diverse as life itself.

For the simplest forms of life, the protists, it might seem that food-getting mechanisms would be the least complex. Indeed, this is probably the case. That food-getting mechanisms at this level can be regarded as simple, however, is not accurate. On the contrary, it would appear from data obtained from a variety of unicellular as well as multicellular organisms, that nutrient acquisition at the molecular level is somewhat complex, and it is clear that the notion that all vital cell nutrients are obtained by simple diffusion processes can be discarded.

Rather, there exist specialized membrane "sites" which are particularly adapted to effect the passage of nutrients into the cell. Thus, they represent food-getting mechanisms at the molecular level.

Such processes are particularly significant if they also function in a regulatory capacity. By the simple virtue of being selectively permeable, the membrane itself regulates cellular life, but there is an obvious advantage in having the ability not only to select kinds but also to determine quantities of nutrients which pass through the cell membrane. Such advantages are conferred to cellular membranes by the possession of transport sites (sometimes called "carriers") which may be complex entities in themselves.

The unicellular organisms are of special interest since such cells must retain all of the capacities which are allotted only to certain cells of multicellular organisms. Presumably this is reflected in the membrane of unicellular organisms. Such a membrane must be as specialized as the environment requires (or permits). Therefore, the study of functional attributes of the unicellular membrane may allow a direct correlative study of relations between the environment and adaptations of the membrane.

B. Concept of the Carrier and Transport. The movement of substances through cell membranes has been the subject of a rather voluminous literature, and no attempt to review such studies here could be adequate. Although some knowledge on the part of the reader may be presumed, some pertinent relationships will be discussed.

To simplify greatly (and excluding vesicle movements), basically only two kinds of cell membrane translocation are known: 1) passive diffusion and 2) carrier-mediated transport. The first is, as the name implies, a passive process which obeys Fick's Law. When kinetics of a diffusing substrate are examined, it is found that the rate of translocation is directly proportional to the concentration of the substrate under consideration.

The second type of transport exhibits quite different kinetics. It has been found that the rate of passage of some substrates is not linear with respect to concentration but reaches a maximum which cannot be exceeded even at infinite concentrations of substrate. Such kinetics are characteristic of any adsorption process, and it is inferred that an adsorption event does occur.

It has been increasingly appropriate to assume that transported substances bind¹ reversibly with a membrane component, called the "carrier" which facilitates membrane translocation (see for review: Wilbrandt and Rosenberg, 1961). Thus the relationship can be formulated:

where E represents the carrier and S is the transported substrate and ES is the carrier-substrate complex.

¹The nature of the bond, i. e., covalent, ionic, or hydrogen, is not important to the concept nor is the length of time in which the bond exists.

There are generally two kinds of mediated transport:

1) facilitated diffusion and 2) active transport ².

Active transport will not be considered at this time since there is no evidence that it is involved with the system described in this study.

Consider the hypothetical situation for a transported substrate:

The relationships for the rate of substrate transfer from one side of the membrane to the other can be formulated:

$$v = V_{\max} \left(\frac{S_o}{S_o + K_m} + \frac{S_i}{S_i + K_m} \right)$$

where the subscripts "o" and "i" refer to the substrate concentrations outside and inside of the cell membrane (Wilbrandt and Rosenberg, 1961).

² These terms are widely used but their definition is not universally equal. In view of recent data on the co-transport of sodium and glucose in the hamster intestine (Crane, 1965; Stein, 1967) both mechanisms are considered basically identical with the exception that in active transport, the facilitated diffusion mechanism is coupled to the active extrusion of sodium ions with the consequent accumulation of substrate.

This equation shows that the rate will depend on the ratio of $S_o:S_i$. It is important to recognize that the entry of many molecules can be defined by such simple algebraic expressions, and they describe, albeit somewhat superficially, the molecular consequences of membrane transport. With these expressions, the transport behavior of a variety of adsorbed substances can be compared via the kinetic parameters K_m ³ and V_{max} .

It is apparent from the above general transport equation, that as S_i becomes larger, it approaches S_o and

$$\frac{S_o}{S_o + K_m} - \frac{S_i}{S_i + K_m}$$

approaches 0 and therefore, when $S_i = S_o$, velocity = 0. That is, if no other factors are involved, the transport system will come to equilibrium and no net transfer of substrate can be detected.

On the other hand if it is assumed that S_o is very much larger compared to S_i , a great simplification can be made. In this case the term $S_i/(S_i + K_m)$ can be neglected and the equation becomes:

$$v = V_{max} \left(\frac{S_o}{S_o + K_m} \right)$$

³ The kinetic constant K_m which is the concentration at which the observed reaction rate is half maximal, must be regarded with caution. As has been discussed in detail by Webb (1963) and Crane (1960), the K_m is not necessarily a measure of "affinity" (the reactivity) between a substrate and an enzyme.

which is the same form as the equation derived by Michaelis and Menten (1913) to describe the kinetics of enzyme-catalyzed reactions. That S_i is very small compared to S_o can be assumed only in initial rate experiments where measurements are concluded before significant amounts of substrate appear on the inside or if another metabolic step, i.e., the phosphorylation of glucose, is so rapid that significant amounts of free substrate cannot accumulate.

C. Transport and Its Relation to Metabolism. As has been discussed, mediated absorption of nutrients is a food-getting mechanism at the molecular level and its importance is thus established. However, it is obvious that the significance of mediated systems cannot be interpreted without some knowledge of the events which occur subsequent to membrane translocation. It must be determined what the transport of a given substrate means in terms of the overall metabolism of the organism. Does transport affect the supply of a metabolizable substrate? Does it regulate osmotic balance? Such questions are obviously important. It is necessary, therefore, to have some understanding of how a transported substrate may be acted upon once it reaches the cell interior. Since the present study examines the nature of carbohydrate transport in Trypanosoma gambiense, it is of great importance to review briefly the carbohydrate metabolism of this organism.

Trypanosoma gambiense appears to oxidize glucose

only as far as pyruvic acid (von Brand, 1966; Danforth, 1967)⁴. It has been observed that small quantities of glycerol are also formed under certain conditions, but its contribution is small compared to pyruvate production. It has also been observed that molecular oxygen is rapidly consumed along with glucose in a one to one ratio (Ryley, 1956). It has been established that the oxidation of glucose occurs through the Emden-Meyerhof-Pathway as far as pyruvate, and the overall relationship can be formulated:

In this scheme, it has been found that the reoxidation of reduced coenzyme (NADH) is accomplished by the coupling of glucose oxidation with an L α -glycero-phosphate dehydrogenase system which can be shown schematically (Danforth, 1967):

⁴The literature on trypanosome carbohydrate metabolism is extensive but has been reviewed particularly well by von Brand (1966) and Danforth (1967) and these are given as references to the general outline of trypanosome metabolism presented here.

Clearly the energy metabolism exhibited by T. gambiense is greatly abbreviated compared to many other cell types which metabolize glucose completely to carbon dioxide and water. The observations (Ryley, 1956) indicating that this parasite does not utilize the tri-carboxylic acid cycle to any significant extent is in line with this view. It would appear, therefore, that the two moles of ATP which are presumably generated when glucose is oxidized by way of the Emden-Meyerhof-Pathway are sufficient to support the life processes of these flagellates. Since it has been shown that the blood form of the African trypanosomes have little or no stored polysaccharide (von Brand, 1966), the importance of an available metabolizable substrate is paramount. Indeed many workers have observed that these trypanosomes can survive only a few minutes in the absence of an appropriate substrate. Therefore it can only be inferred that the membrane translocation of glucose or other suitable substrates is of primary importance in the energy metabolism of T. gambiense.

D. The Present Study. It has been shown that, of those tested, Trypanosoma gambiense metabolizes only four carbohydrates: glucose, mannose, glycerol and fructose (von Brand, 1966). Seed et al (1965) studied the effects of various glucose analogues as inhibitors of oxygen consumption in T. gambiense. It was found that 2-deoxyglucose inhibited O₂ uptake (as well as acid production) when glucose, mannose, glycerol or fructose were substrates. No inhibition

by 2-deoxyglucose was observed when cell lysates, as opposed to whole cells, were utilized in their experimental design. It was suggested that inhibition might occur at a membrane transport step.

Von Brand et al (1967) studied the metabolism of glucose and glycerol in the culture form of nine species of trypanosomes. They observed that the culture form of T. gambiense removed more glycerol than glucose from a medium containing equal quantities of both substrates, but they found that the total carbon removed from the incubation medium was equal to that removed when either substrate was offered singly. This, they suggested, indicated that hexoses and glycerol share a common transport locus.

The studies of von Brand et al (1967) and Seed et al (1965) suggested that the interaction of carbohydrates at the membrane was more complex than formerly indicated. The present study was undertaken to examine in more detail the nature and specificity of carbohydrate absorption in Trypanosoma gambiense. Evidence will be presented that 1) the absorption of glucose, mannose, glycerol and fructose does not occur by simple diffusion, but rather by carrier-mediated facilitated diffusion; 2) the absorption mechanism is physiologically adapted to the trypanosome's environment; 3) appropriate kinetic analysis reveals that simple assumptions for the molecular events involved for carbohydrate transport in T. gambiense are not justified; 4) the several transported carbohydrates share a common transport mechanism although they exhibit different affinities for it.

MATERIALS & METHODS

A. The Organism. Trypanosoma gambiense was described by Sir David Bruce (1911) who noted that the morphology of this hemoflagellate was not uniform, and he presented illustrations of forms which ranged from "long and slender" (LS forms) to "short and stumpy" (SS forms). Intermediate forms were easily recognized. Such polymorphism is typical of any freshly isolated strain of T. brucei [synonyms: T. gambiense and T. rhodesiense; (Omerod, 1967)], even if cloned from a single trypanosome.

A serious problem arose when it was discovered that syringe-passaged strains soon became monomorphic. Under such circumstances only LS forms were found, and some workers noted a concomitant increase in virulence and susceptibility to arsenical drugs (Browning & Guibransen, 1935; Murgatroyd and York, 1937). This problem was investigated in more detail by Goble, et al (1959). They showed that some strains of trypanosomes, including the Wellcome TS strain, remained "relatively stable throughout a number of years" but others did not. They concluded, "prolonged syringe passage in mice does not inevitably lead to inordinate sensitivity to either arsenicals or other drugs". Even now the problem remains unresolved, but the point is made that syringe-passaged trypanosomes may actually evolve in the laboratory, and an old laboratory strain

may not be identical with the organisms originally isolated. Therefore, strict standardization of trypanosome material is required, and one must be cognizant of strain variations.

The Wellcome TS strain of Trypanosoma gambiense was used throughout the study and was obtained in January, 1968, from Dr. J. R. Seed, Tulane University. It is an old laboratory strain and was originally isolated from a sleeping sickness patient at the London Hospital for Tropical Diseases in 1921. Since then it has been mechanically passaged in a variety of rodents in a number of laboratories.

B. Host Animals. The TS strain of T. gambiense readily infects rats. Trypanosomes were harvested from the blood of large (200-300 g) male albino rats obtained from the Holtzmann Rat Company, Madison, Wisconsin.

C. Anesthesia. Chloroform was used to anesthetize rats for inoculation and extraction of infected blood. Early in this investigation it was determined that chloroform anesthesia produced no demonstrable effects on glucose transport rates of T. gambiense.

D. The Infection. The TS strain of T. gambiense produces a highly virulent infection in mice and rats. Such infections have been described by Taliaferro (1941) and are characterized as having a short lag period which is succeeded by a phase of exponential multiplication which is only terminated by the death of the animal.

The length of the infection is largely determined by the size of the inoculum, and the time of peak parasitemia (just prior to death) is highly predictable.

E. Maintenance of Parasites. It is well established that under certain conditions, trypanosomes, as well as some other Protozoa, can survive freezing and prolonged storage at low temperatures (Coggeshall, 1939; Fulton et al, 1953; Manvill, 1943; Saunders and Scott, 1947; Levine and Marquardt, 1955; Allain, 1964; Cunningham, 1963). Lumsden (1967) has pointed out that a "stabilate" (preserved biological material prepared in large quantities) will provide a uniform source for experimentation. In order to facilitate laboratory procedures and standardize infections, a T. gambiense stabilate was prepared employing the techniques of Allain (1964), with modifications. Glycerol was used as a cryoprotectant.

A 20% (V/V) glycerol - KRT (Krebs-Ringer-Tris-maleate buffer - see Appendix A) was prepared, and one ml of this solution was dispensed into each of 48 screw cap test tubes. Several T. gambiense-infected rats were bled by cardiac puncture. Approximately 10 ml blood were withdrawn from each rat. The infected blood was pooled and one ml was delivered into each of the tubes containing glycerol-KRT solution. This gave a final glycerol concentration of 10% (V/V). The tubes were placed in a Virtis Model 11-200 Slow-Freezing Bath (Virtis Company, Inc.). Ninety-five percent ethanol was used as the

coolant. The temperature of the freezing solution was lowered automatically from an initial 10 C to a final -48 C at a rate of 1-2 degrees/minute. The tubes were stored at -80 C until used.

This process was repeated once a year over a four year period to provide sufficient inoculation material for experiments and to insure against aging and loss of infectivity. Periodically tubes were withdrawn and checked microscopically for viability. After four years of study, trypanosomes from tubes frozen in the first year were still motile although the percentage of survival had decreased (10-40%). Infected blood frozen a year or less gave trypanosome survivals of 50-80%.

When a new stablate was prepared, rats were infected with thawed blood from the first stablate to minimize the number of generations between stablate #1 and #2, #3 and #4. In this way, the 'quality' of this strain of T. gambiense was held constant during the period of this investigation.

F. Infections. Forty-eight hours prior to an experiment, a tube of frozen infected blood was withdrawn from the freezer and thawed under cool running tap water. Approximately 0.1 ml was inoculated into the heart of each of six rats.

G. Collection & Preparation of Trypanosomes. Forty-eight hours after infection, six rats were bled by cardiac puncture into 1.0 ml heparinized saline. Nine ml were taken from each rat.

The blood was centrifuged at 1,300 g for 5 min at 2-4 C after which the plasma and trypanosome layers were removed and recentrifuged at 1,300 g for 15 min. The supernatant plasma was discarded, and the trypanosomes were resuspended in 8.0 ml glucose-KRT and centrifuged at 1,300 g for ten minutes. The supernatant fluid was discarded, the pellet resuspended in 8.0 ml glucose-KRT and recentrifuged at 1,300 g for 10 min. The final washed pellet of trypanosomes was resuspended in 34 ml KRT containing no glucose at 37 C. The fluid suspension was distributed immediately into experimental test tubes previously prepared.

Erythrocytes were always less than 5/1,000 trypanosomes in the final trypanosome suspension.

H. Limitations of Experimental Design. It is well known that the blood stream form of the African trypanosomes can survive only a few minutes without the presence of a metabolizable substrate such as glucose, and that the rate of metabolism is quite high (von Brand, 1966). Therefore all preparative and experimental procedures other than actual incubations were conducted in the presence of glucose or fructose at no less than 1.0 mM.

Only twenty-four test tubes could be employed in a single experiment because of the space limitations of the refrigerated centrifuge. Each test tube contained one ml of the desired ^{14}C -substrate plus inhibitor (if called for) at twice (2X) the concentrations to be tested. In most cases, four replicates for each experimental condition were examined.

I. Incubations. At the initial time of an experiment, 1.0 ml (6-9 mg dry wt) of the final washed trypanosome suspension was rapidly dispensed by automatic pipette into each of the 24 tubes. The concentration of reactants was then 1X with a total volume of 2.0 ml. The tubes were incubated in a water bath for 2 min at 37 C. The incubations were terminated as described by Sanchez & Read (1969). This was accomplished by the rapid addition of 8 ml (4 volumes) of cold glucose-KRT to each test tube and immediate centrifugation at 2-4 C for 10 minutes at 1,300 g. The supernatant was discarded and the trypanosomes were resuspended in 8.0 ml cold glucose-KRT. Trypanosomes were checked at this time for motility; in most experiments 95% of the trypanosomes were highly motile at the end of the first wash, but in experiments involving strong inhibitors, motility was lost long before the end of the post-incubation procedures (see RESULTS).

The suspensions were centrifuged for an additional 10 min at 1,300 g, and the resulting pellet was extracted overnight with 70% ethanol.

J. Determination of Radioactivity. The radioactivity of alcohol extracts was determined on dried aliquots in a Nuclear-Chicago gas flow counter.

K. Reactants & Substrates. All ^{14}C -substrates were obtained from New England Nuclear Corporation, Boston, Massachusetts.

These were diluted with KRT and carrier substrate and incubation media contained specific activities ranging from 0.01-0.10 μ curies/ μ mole. Non-radioactive substrates were of reagent grade and obtained from commercial sources.

RESULTS

A. The Significance of Isotopic Extraction. It is important to recognize that the radioactivity of the ethanol extracts may be in compounds other than glucose. The metabolic rate of T. gambiense is quite high (Danforth, 1967), and it is reasonable to expect that much of the ^{14}C -glucose absorbed will be metabolized in two minutes. The nature of the ^{14}C -metabolite is secondary, however, since the recovery of each ^{14}C -molecule from within the cell represents an absorbed glucose molecule. Therefore, if the transported ^{14}C -substrate is metabolized, the kinetics of absorption will not be altered as long as the metabolic products remain within the cell and are ethanol-soluble.

B. The Absorption of Glucose. Trypanosoma gambiense absorbed glucose rapidly from the incubation medium (Figure 1). As has been observed in a variety of cell types and tissues (Stein, 1967), the uptake of ^{14}C -glucose was not linear with respect to glucose concentration and appeared to follow saturation kinetics. As glucose concentration increased, the velocity of uptake increased to a decreasing extent, and the system was essentially saturated at concentrations in excess of 5 mM. These kinetics, which are typical of facilitated mechanisms when initial rates are studied (Wilbrandt & Rosenberg, 1961)

are described quite satisfactorily by the Michaelis-Menten equation:

$$\text{Velocity (of transport)} = \frac{V_{\max} S}{K_m + S}$$

where $V_{\max}$ = maximum velocity at infinite substrate concentration, and K_m is that concentration of substrate at which the velocity is half maximal (Michaelis and Menten, 1913). This does not imply that glucose transport is an enzymatic process, for such kinetics are characteristic of any adsorption phenomenon. However, it is implicit, when such kinetics are observed, that the amount of material available for adsorption is limited. In this case, the number of sites for glucose transport is limited.

Linear regression analysis of the data from Figure 1 yielded the linear relationship depicted in Figure 2. The maximum velocity ($V_{\max}$) for glucose transport was found to be 47.9 moles/g dry wt/2min, and the K_m was 1.35 mM.

As described in MATERIALS & METHODS, the post-incubation procedures are somewhat lengthy and the question arises as to how much ^{14}C -glucose uptake occurs during these operations. Any adsorption after the theoretical termination of an incubation period would be a source of error and would give a somewhat higher value for sugar transport. Figure 3 compares the rate of glucose entry when the incubation time was 2 min or 0. The lower curve in Figure 3

demonstrates the degree of efficiency with which the absorption of glucose can be terminated by the post incubation procedures employed. That some residual uptake occurred is not unexpected and is a source of error, albeit small.

It is important to note that the absorption curve for zero time cannot be subtracted from the 2 min curve as a simple correction factor, for it is not known if post-2 min-incubation absorption is equal to absorption when incubation time is zero. Therefore, the control curve in Figure 3 is probably only a close approximation of post-incubation sugar absorption; and since it is relatively small, the error is considered acceptable.

C. Variation and Reproducibility. Some day-to-day variation in the velocity of sugar transport for T. gambiense was observed. Table 1 shows the rate of glucose uptake in 5 individual experiments. Each value is the mean of four replicates. It may be observed that, while values were fairly reproducible between experiments, significant variation could occur between any two experiments. Although this variation was not particularly great, the variation within a single experiment was usually considerably less, as evidenced by Table 2 showing the uptake of glucose at 2-4 C. Therefore, the appropriate controls were always included so that all comparisons could be made within the same experiment. This procedure insured maximum reproducibility.

Table 1. Variation in Rate of Glucose Uptake in 5 Experiments

Concentration of glucose	μ moles glucose/g dry wt/2 min					Mean $\pm$	S. D.
	1	2	3	4	5		
10.0 mM	28.4	30.9	36.2	30.7	29.6	21.2	2.97
5.0 mM	22.9	26.7	29.9	29.3	27.8	27.3	2.76
2.5 mM	18.7	23.3	23.5	24.3	23.8	22.7	2.25
1.0 mM	14.6	18.5	18.4	17.7	15.6	17.0	1.73
0.5 mM	11.3	13.0	12.6	13.7	12.3	12.6	0.85
0.1 mM	2.6	3.2	2.7	2.6	2.4	2.7	0.15

Table 2. Uptake of ^{14}C -glucose at 2-4°C in a Single Experiment

Glucose	μ moles	μ moles	μ moles	μ moles	Mean $\pm$	S. D.
.1 mM	.40	.37	.31	.38	.36	0.04
.5 mM	.96	.82	.87	.83	.87	0.06
1.0 mM	1.64	1.34	1.34	1.66	1.49	0.18
2.5 mM	2.98	2.46	2.93	2.92	2.82	0.24
5.0 mM	4.92	4.24	4.68	5.03	4.71	0.35
10.0 mM	7.52	7.40	7.98	8.25	7.79	0.40

D. Effect of Temperature. Since many of the experimental procedures were carried out in the cold, the effect of such temperatures upon the absorption of glucose and fructose was determined. The uptake of glucose and fructose at 37 and 2-4 C is shown in Figure 4. It is immediately apparent that the transport of these substrates is greatly reduced by the lower temperature range. Using the uptake rates shown for 10 mM substrate in Figure 4, a Q_{10} temperature coefficient can be calculated. The Q_{10} for glucose transport was 1.38 and for fructose transport 1.42. The significance of these values will be considered later, but it should be pointed out that different Q_{10} 's result if the effect of temperature upon the absorption of glucose and fructose is determined at other substrate concentrations. Further examination of Figure 4 reveals that decreasing the temperature not only reduces the absorption of glucose and fructose but also changes the shape of the uptake curves, i. e., alters the absorption kinetics. As a consequence depending upon the concentrations of substrates chosen, calculated Q_{10} values range from 1.38 to nearly 2.

E. The Absorption of Other Substrates. The uptake of some related monosaccharides and glycerol by T. gambiense was examined. The uptake of glucosamine, fructose, mannose, 2-deoxyglucose (D-2-D), and glycerol are shown in Figures 5 and 6. Like glucose, these substrates were absorbed by what appeared to be a carrier-mediated process since saturation kinetics were observed.

The calculated apparent K_m and V_{max} for these substrates are given in Table 3.

Table 3. Apparent K_m 's and V_{max} 's
For Some Absorbed Carbohydrate Substrates

Substrate	K_m^*	V_{max}^{**}
Glucose	1.35	47.9
Fructose	1.58	20.1
Glucosamine	1.20	33.5
Mannose	0.84	48.3
Glycerol	0.60	39.7
2-Deoxyglucose	0.16	30.4

* K_m is in millimoles/liter

** V_{max} is in μ moles substrate/g dry wt/2 min

It has been reported that 2-deoxyglucose inhibits O_2 consumption in the T.S. strain of T. gambiense (Seed et al, 1965). A simple experiment to determine the suitability of this sugar as an energy substrate is to incubate trypanosomes in a 2-deoxyglucose solution at 37 C and check microscopically for trypanosome motility after various time intervals. After a two minute incubation of T. gambiense with 10 mM D-2-D, trypanosome motility was significantly reduced as

compared with organisms incubated in 10 mM glucose. After a five minute incubation, irreversible loss of motility occurred in 90% of the 2-deoxyglucose-incubated organisms. This was taken as evidence that D-2-D was not appreciably metabolized by T. gambiense.

The absorption of 2-deoxyglucose is shown in Figure 6. The kinetics for its uptake were quite similar to those for glucose. The glucose analogue, 3-O-methylglucose, was not taken up by T. gambiense to an appreciable extent in 2 min.

F. Competition Among Substrates. That the above mentioned carbohydrates are absorbed through a carrier-mediated process is indicated by their absorption kinetics. Such information alone, however, is not sufficient to decide whether all substrates share a common site, or if each is taken up through a different mechanism. A simple method for showing that substrates utilize a common locus is the demonstration of mutual competitive inhibition.

Like adsorption kinetics, competitive inhibition is not limited to enzyme-catalyzed reactions and has been demonstrated in a variety of transport systems (Stein, 1967). By also binding reversibly with a particular transport site, the presence of such an inhibitor increases the apparent K_m of the site for its substrate while the V_{max} is not altered.

Competitive inhibition is most often exhibited between structurally analogous compounds, e. g., the well known malonate inhibition of succinic dehydrogenase. The carbohydrates which have

been shown here to be transported by T. gambiense are certainly structural analogues, and it is no surprise that competitive inhibition among these substrates was found.

The inhibitory effect of 2-deoxyglucose (D-2-D) on the uptake of 2.5 mM ^{14}C -glucose is shown in Figure 7. As the concentration of D-2-D increased, the rate of glucose uptake decreased rapidly at first but more slowly at higher D-2-D concentrations. At an inhibitor to substrate (I/S) ratio of 16:1 (40 mM:2.5 mM), an inhibition of $87 \pm 3\%$ was obtained. This pattern of inhibition was typical for competing substrates as is indicated in Figures 8-12. In all cases, the nature of inhibition of one substrate on another was determined to be competitive by the Lineweaver-Burke method of plotting (Webb, 1963).

Examination of Figure 8 shows the effect of mannose, glycerol, glucosamine, and unlabeled glucose on ^{14}C -glucose absorption as a function of inhibitor concentration. The effect of cold glucose is particularly important since it should be a good competitive inhibitor of its own absorption. By inspection, the shape of the curve is hyperbolic and is typical for competitive inhibitions plotted by this method (Webb, 1963). The amount of inhibition appears to become asymptotic at less than 100%. This unexpected but important point will be analyzed in more detail in the DISCUSSION.

Although the maximum inhibition is less than 100%, the effect of cold glucose on ^{14}C -glucose absorption can be used as a reference point for comparing the effects of other substrates. For

example, Figure 8 shows the effect of increasing mannose concentrations. The mannose curve is quite similar to the cold glucose curve, and we may conclude that mannose, like unlabeled glucose, competes for and binds with the glucose transporting mechanism.

The shape of the glycerol inhibition curve in Figure 8 is similar to that of mannose, but one cannot tell by inspection if the maximum degree of inhibition will be as great as exhibited by mannose or cold glucose. The effect of glucosamine on glucose absorption appears to be different in that over the concentration range tested, glucosamine was not as effective an inhibitor of glucose uptake as mannose or glycerol.

The inhibition of fructose uptake is shown in Figure 9. It may be noted that the inhibition curves resemble each other closely. This is especially true for glycerol, glucose and mannose suggesting that the mode of inhibition may be identical.

Figure 10 shows the inhibition of 2-deoxyglucose absorption by glucose and glycerol. In Figure 11, it may be seen that mannose uptake is inhibited strongly by glucose, glycerol and 2-deoxyglucose. Similarly, Figure 12 represents the effective inhibition of glycerol absorption by mannose and glucose.

In Figure 13, however, the pattern of inhibition is quite different. This figure shows the effect of fructose on the absorption of glucosamine, glucose, mannose, D-2-D, and glycerol. At an I/S ratio of 1:1 and 2:1, fructose fails to inhibit all substrates with the

exception of glucosamine; however, when fructose concentration reaches 20 mM, glucose, mannose, glycerol and D-2-D are all inhibited to some extent; at an I/S of 16:1, inhibition by fructose is nearly 40% for glucose.

The four metabolized substrates were tested for mutual inhibition in all possible combinations of pairs. These data are depicted in Figures 8-12 and are summarized in Figure 14. The values expressed represent the percent inhibition obtained when the I/S ratio was 16 (4 when fructose was the inhibitor). It is particularly important to note the degree of inhibition obtained when an unlabeled substrate was tested against its ^{14}C -counterpart. As already indicated, it is obvious that all molecules of the same species, whether labeled or unlabeled, compete for and bind with the same transport site; therefore, the inhibitory effects of one carbohydrate upon another in Figure 14 must be compared with results obtained when unlabeled substrate is added to labeled substrate of the same species. For example, Figure 14 shows that mannose inhibits glucose absorption 86%. Since this differs very little from the glucose- ^{14}C -glucose inhibition, it may be inferred that mannose is as an effective competitor as glucose for the glucose transport site at this particular I/S ratio (16). Likewise, glucose inhibits mannose absorption by 83%. It is clear that mannose and glucose show mutual inhibition, and such evidence is used to conclude that these two sugars are absorbed by the same mechanism (Lefevre, 1961; Crane, 1960; Stein, 1967).

The interaction between glucose and glycerol and mannose and glycerol is somewhat different. We see from Figure 14 that when glycerol interacts with glucose or mannose at an I/S of 16, the average inhibition is around 62%. As was mentioned earlier, all of these inhibitions are competitive; therefore, the reason for the smaller degree of inhibition is not immediately obvious unless the different carbohydrates have different affinities for the same site; nevertheless, that these substrates share the same transporting mechanism is indicated by the mutual inhibition exhibited.

Table 4 shows the effect of raising the I/S ratio on the glucose-mannose-glycerol interaction. It is clear that greater inhibition can be achieved by raising the I/S ratio which indicates that a greater degree of competition for the same site can be achieved.

Table 4. Effect of Increasing Inhibitor to Substrate Ratios On Inhibition of Substrate Absorption

¹⁴ C-substrate	Inhibitor	I/S Ratio	% Inhibition
Glucose	Glycerol	16:1	65
Glucose	Glycerol	40:1	77
Mannose	Glycerol	16:1	64
Mannose	Glycerol	40:1	78
Glycerol	Glucose	16:1	56
Glycerol	Glucose	40:1	69
Glycerol	Mannose	16:1	63
Glycerol	Mannose	40:1	73

The situation is more complex with respect to fructose, for strong mutual inhibition does not exist. While fructose absorption is strongly inhibited by glucose, mannose, and glycerol (Figure 9), the reverse is not true (Figure 13). In Figure 14, for example, at an I/S ratio of 16, glucose, mannose and glycerol inhibit fructose uptake 91, 92 and 86% respectively (Figure 14). The reciprocal experiments show that 10 mM fructose exerts an inhibition of less than 10%. The low I/S ratio of 4 (10 mM fructose:2.5 mM substrate) was chosen here for technical reasons. It is clear from Figures 13 and 14 that fructose is a poor inhibitor of all but glucosamine transport.

In other experiments, I/S ratios of fructose to glucose were tested up to 100. The maximum inhibition obtained was no greater at an I/S ratio of 50 than at 100 and never exceeded 45%. Still, the nature of this inhibition, as is true for the interaction of the other substrates, was found to be competitive as evidenced by Figure 15.

The interaction of glucosamine with the other substrates should be considered in more detail at this time. Its effect on the uptake of glucose and fructose transport is shown in Figures 8, 9, 14-A and 16. Examination of Figure 8 reveals that glucosamine is the least potent inhibitor (other than fructose) of glucose transport. On the other hand, glucosamine inhibits fructose absorption almost as effectively as glucose (Figures 9 and 14-A). In Figure 16 it is evident that glucosamine is a much more potent inhibitor of fructose than of glucose absorption - an important point to later discussions. Reciprocal

experiments reveal that although glucose uptake is inhibited less by glucosamine than is the uptake of fructose, glucose is more potent than fructose as an inhibitor of glucosamine uptake (Figures 17 and 18). These results suggest that in order of decreasing ability to exert inhibition of transport, glucose > glucosamine > fructose.

The effect of glucose and/or fructose on the uptake of ^{14}C -glucosamine is examined in Table 5. It is evident that the combined inhibition by glucose and fructose is not significantly greater than the inhibition produced by glucose alone.

Table 5. Effect of Glucose and/or Fructose
On ^{14}C -glucosamine Absorption

Glucosamine	Glucose	Fructose	% Inhibition
2.5	0	0	0
2.5	20	0	88.8
2.5	0	20	63.2
2.5	20	20	90.2

The range of inhibition values is within 5.

That the patterns of mutual inhibition are somewhat complex gives rise to some initial confusion concerning the processes involved. For this reason, a brief consideration of the mechanisms which may account for the inhibition phenomena which have been described in this section is now in order.

Two hypotheses can be suggested to account for the inhibition data. The first hypothesis assumes the existence of two transport sites: a glucose site and a fructose site. As has been shown, mannose, glucose, and glycerol exhibit relatively strong mutual inhibition for one another. This suggests that these sugars are absorbed through a common locus (the glucose site). Fructose does not greatly inhibit the transport of these compounds at fixed I/S ratios (Figure 14). The failure of fructose to inhibit glucose, mannose and glycerol to any great extent at low I/S ratios (Figure 13) may suggest that fructose does not utilize the glucose site exclusively but rather is also absorbed through a similar yet different mechanism. The strong inhibitory effects of the other sugars on fructose absorption is accounted for if these sugars react with the second site while not actually being translocated by it. Since glucosamine exhibits mutual inhibition with fructose as well as glucose, this sugar, according to this hypothesis, must be absorbed through the second site while reacting with but not being transported through the glucose site.

An alternative hypothesis assumes the existence of only one transport site which mediates the absorption of all of the transported carbohydrates studied. The various patterns of inhibition kinetics exhibited between pairs of carbohydrates can be explained, according to this hypothesis, by differences in relative affinities of the several carbohydrates for the transport mechanism.

Without further considerations at this time, a definite choice between these two hypotheses is premature; however, it should be mentioned that, for reasons to be pointed out in the DISCUSSION, the second alternative is perhaps more likely.

G. Specificity of Glucose Absorption. From the information presented so far, one might conclude that the glucose transporting mechanism is rather nonspecific since it is utilized also by mannose, glycerol, D-2-D, and possibly glucosamine and fructose. Table 6, however, reveals that the specificity of glucose and fructose uptake is greater than expected. Here a large number of carbohydrates were tested as inhibitors of glucose or fructose transport. The I/S ratios were 10 (10 mM:1 mM) except when fructose was the inhibitor when the I/S ratio was 4. An examination of Table 6 reveals that very few⁵ carbohydrates inhibited glucose or fructose transport, and most of those that were inhibitory have already been described above. It is interesting that in Table 6, several glycerol analogs failed to inhibit glucose uptake including 1,3-propanediol (2-deoxyglycerol) and 1,2-propanediol (1-deoxyglycerol). Propanol and ethylene glycol were also without effect.

⁵This is not to say, of course, that some of these compounds may have such a low affinity for the glucose site(s) that an I/S ratio of 10 is not sufficient to indicate inhibition.

Table 6. The Effect of Several Carbohydrates On The Uptake of 1 mM ^{14}C -glucose and 1 mM ^{14}C -fructose

Inhibitor	Glucose	Fructose
A. Glycerol analogs		
1,3-Propanediol	—	—
1,2-Propanediol	—	—
1-Propanol	—	—
Lactic acid	—	—
Propionic acid	—	—
Glycerol	+	+
B. Glucose analogs		
D-Allose	—	—
D-Galactose	—	—
L-Fucose	—	—
D-Sorbitol	—	—
D-Mannitol	—	—
D-Gluconic acid	—	—
D-Gluconic acid lactone	—	—
Glucuronic acid	—	—
Glucuronic acid lactone	—	—
α -Methyl-D-glucoside	—	—
Phenyl-D-glucopyranoside	—	—
3-O-Methylglucose	—	—
D-Glucose	+	+
D-Mannose	+	+
D-Glucosamine	+	+
N-Acetyl-D-glucosamine	+	+
2-Deoxy-D-glucose	+	+
C. Fructose analogs		
D-Tagatose	—	—
L-Sorbose	—	—
D-Fructose	+	+
D. Disaccharides		
Cellobiose	—	—
Lactose	—	—
Maltose	—	—
Sucrose	—	—
E. Miscellaneous		
Inositol	—	—
Ethylene glycol	—	—
D-Arabinose	—	—

Table 7. The Effect of Some Inhibitors on Glucose Transport and Motility of Trypanosoma gambiense

Inhibitor	Concentration	Effect on Transport in 2 Minutes	Effect on Motility*		
			5 Min	15 Min	30 Min
NaAsO ₂	2×10^{-4} - 4×10^{-1} mM	None	-	+	+
NaF	10^{-2} - 20 mM	None	-	+	+
CuCl ₂	10^{-4} - 1 mM	None			
NaCitrate	10^{-4} - 1 mM	None			
2, 4-Dinitrophenol	10^{-3} - 10^{-1} mM	None	-	-	-
Ouabain	10^{-4} - 10 mM	None			
Uranyl NO ₃	10^{-5} - 10^{-1} mM	None			
Iodoacetic acid	10^{-3} - 10 mM	Inhibition	+	+	+
HgCl ₂	10^{-6} - 1 mM	Inhibition	+	+	+

* Each inhibitor was tested against 1.0 mM ¹⁴C-glucose. Incubation time for determination of transport effects was 2 min. Motility assays were determined separately and assayed by microscopic examination at regular time intervals. In the motility assays, glucose was present in experimental and control solutions at 2.5 mM while inhibitor concentration was 0.1 mM. A "+" indicates that motility was reduced in the time interval shown.

Of the glucose and mannose analogous tested, even those which differed from glucose at only a single carbon position were not inhibitory. For example, D-allose varies from glucose only at carbon 3 but did not affect transport. The importance of the number 3 carbon is further emphasized by the fact that experiments mentioned above showed that 3-O-methylglucose was not absorbed by T. gambiense in 2 min.

D-mannitol and D-sorbitol were also without effect. These sugar alcohols structurally resemble mannose and glucose respectively except at carbon 1 where the aldehyde is replaced with alcohol and hydrogen groups. Similarly gluconic acid, α -methylglucoside and phenylglucospyranoside were not inhibitory. The importance of carbon 6 is suggested by the inability of gluconic acid to affect uptake. That galactose did not cause inhibition indicates that specificity at carbon 4 is great since this aldose is different from glucose only at this carbon.

It is interesting that several disaccharides were without effect. Also, the ketoses, tagatose and sorbose, did not affect the transport of glucose or fructose.

The sugars which inhibited glucose and fructose transport have a common characteristic. Each differ from glucose in configuration or substitution only at carbon 2. Mannose, for example differs from glucose only in configuration of the hydroxyl group around the number 2 carbon. Similarly, the glucosamine molecule is structurally identical to glucose except for the substitution of an amino group for the hydroxyl group at carbon 2. The same argument can be applied to

2-deoxyglucose. In this case, 2-deoxyglucose (which is also 2-deoxymannose) has no hydroxyl group at position 2.

Table 6 indicates that N-acetylglucosamine (NAG) inhibited glucose and fructose absorption. Its effect on glucosamine absorption is indicated in Figure 18, and other experiments have demonstrated that this inhibition was competitive. The kinetics of NAG uptake in the absence or presence of glucosamine is shown in Figure 19. No saturation kinetics for NAG transport were observed in the concentration range tested; the uptake of NAG, rather, obeyed Fick's Law. That NAG entered by diffusion is further emphasized by the fact that glucosamine failed to act as a competitive inhibitor of NAG transport.

H. Effect of Metabolic Poisons and Other Inhibitors.

The use of metabolic poisons and other inhibitors has often aided in the elucidation of some types of cellular mechanisms. In particular, an organism's dependence upon certain metabolic pathways can be shown by the selective use of specific inhibitors. Unfortunately many anti-metabolic poisons, such as the heavy metals, are rather nonspecific. The use of nonspecific inhibitors, however, is sometimes useful when it is found that a cellular process can be distinguished only on the basis of sensitivity to such an inhibitor.

Several well known inhibitors were tested for inhibition of ^{14}C -glucose absorption by T. gambiense, and this information

is summarized in Table 7. These compounds have been shown to have strong effects on other systems, but only a few of these have been tested as inhibitors of T. gambiense metabolism. For example, von Brand et al (1953) reported that sodium arsenite reduced O₂ uptake by the T.S. strain of T. gambiense. Also they found that iodoacetate inhibited O₂ uptake. In this early study, it was suggested that entry of metabolized substrates might be affected. Table 8 confirms this speculation for the effect of iodoacetic acid, but not for NaAsO₂. Sodium arsenite did, however, reduce motility in less than 15 minutes and its effect on motility correlates with the reduced O₂ uptake reported by von Brand.

2,4-dinitrophenol is widely known to uncouple oxidative phosphorylation, and it has often been employed to show an energy requirement for active transport systems. Dinitrophenol did not affect glucose absorption by T. gambiense in 2 minute incubations.

The active absorption of glucose has been linked to the "sodium pump" in at least one system (Crane, 1965; Stein, 1967). The active extrusion of sodium ions (the "sodium pump") has, in turn, been associated with a K⁺ - Na⁺ activated, Mg⁺⁺-dependent ATPase is specifically inhibited by ouabain. Ouabain inhibition of ATPase results in the failure of the cell or tissue to actively extrude sodium ions. With these considerations in mind, ouabain was tested on T. gambiense for its effect on glucose transport. Ouabain was entirely without effect at concentrations as high as 10 mM.

Fluoride ions, CuCl_2 , sodium citrate and uranyl nitrate failed to affect glucose transport. In the time interval tested for motility effects, only F^- decreased trypanosome motility as compared to controls.

Similar to iodoacetate, HgCl_2 was found to be a potent inhibitor of the glucose site. The effects of iodoacetic acid and HgCl_2 are detailed in Tables 8 and 9.

Table 8. Effect of Iodoacetic Acid

Iodoacetic Acid	Mean CPM	$\pm$ S.D.	% Inhibition
0 mM	446.8	24.3	-
10^{-3} mM	276.3	18.8	38.2
10^{-2} mM	203.7	14.2	54.4
10^{-1} mM	166.5	7.2	62.7
10^0 mM	147.0	8.3	67.1
10^1 mM	143.7	4.3	68.8

Table 9. Effect of HgCl_2

Glucose	HgCl_2	Mean CPM	% Inhibition
1 mM	0 mM	443	-
1 mM	10^{-6} mM	442	N.S.*
1 mM	10^{-4} mM	445	N.S.
1 mM	10^{-2} mM	85	81
1 mM	10^{-1} mM	56	87
1 mM	10^0 mM	20	96

*Not significant at 95% level.

The diphenylglycoside, phlorizin, has been known for many years to produce renal glycosuria in mammals by preventing sugar reabsorption in the kidney tubules (LeFevre, 1961). The action of phlorizin has been studied in a number of other tissues including erythrocytes, ascites cells, rat intestine and rat heart muscle (Stein, 1967), and it has become clear that not all transport systems respond to phlorizin in the same manner. The effects of phlorizin are shown in Table 10. Phlorizin (0.2 mM) inhibited the uptake of 1 mM ^{14}C -glucose some 20%; however at 0.02 and 0.002 mM significant inhibition was not observed.

Table 10. The Effect of Phlorizin On
The Uptake of 1.0 mM ^{14}C -glucose

Inhibitor	Mean CPM	S. D.	% Inhibition
0	181.9	7.2	-
0.2 mM phlorizin	145.7	8.8	20

I. Effect of Cation Replacement. The importance of Na^+ ion has been well established in relation to sugar transport by the mammalian intestine (Crane, 1964; 1965) and amino acid transport by ascites tumor cells (Christensen, 1962). In these systems it has been shown that Na^+ ions are required for active transport but not for mediated translocation of these substrates (Stein, 1967). Evidence for a sodium-sugar cotransport system in T. gambiense was

sought by determining the effects of cation replacement.

The composition of KRT buffer used in most experiments is given in Appendix A. Sodium-free KRT was prepared by replacing NaCl with tris-HCl on a molar equivalent basis and adjusting the pH with KOH instead of NaOH.

For technical reasons, the effect of 25 and 50% replacement of Na^+ ions was compared to controls or, 100% and 75% replacement was compared to only 50% replacement. The results were unequivocal. Replacement of Na^+ ions with tris ions did not alter the glucose-absorbing ability of T. gambiense in two minutes. In addition, trypanosomes were incubated (at room temperature) in buffer solution where Na^+ ions were entirely replaced. The motility of trypanosomes was observed microscopically, periodically for two hours. In this time, trypanosomes showed no apparent loss in motility compared with control samples.

The effect of replacement of K^+ , Mg^{++} , and Ca^{++} ions was also determined. In this case, the appropriate ion was replaced with its molar equivalent of NaCl. For technical reasons, 100% replacement was compared to 50% replacement. No effect was observed in two minute incubations.

DISCUSSION

A. Glucose Absorption and Relation to Metabolism.

For many years, it has been known that Trypanosoma gambiense metabolizes glucose (von Brand, 1966) as an energy substrate. The translocation of this monosaccharide across the trypanosome membrane is implicit with its utilization. Rather than being a random or accidental process, the data presented here (Figure 1) indicate that glucose transport is a mediated process: the membrane translocation of glucose is facilitated by its binding to a limited number of sites which subsequently effect the transfer of glucose from the outside to the cell interior. The evidence for this hypothesis is threefold: 1) the actual entry into the cell; 2) the saturation kinetics observed (Figure 1) which follow the algebraic relationship between rate of transport and substrate concentration:

$$v = \frac{V_{\max} S}{K_m + S}$$

or its reciprocal form: $1/v = \frac{1}{V_{\max}} + \left(\frac{K_m}{V_{\max}} \cdot \frac{1}{S} \right)$ (Figure 2)

3) competitive inhibition of ^{14}C -glucose uptake by unlabeled glucose as well as closely similar structural analogues of glucose (Figure 8).

The second and third points distinguish glucose entry from passive diffusion, the kinetics of which obey Fick's Law with respect to substrate concentration, and linear rather than saturation kinetics are observed.

The data presented in this study indicate that the rate of glucose transport is quite rapid at higher sugar concentrations. At 10 mM glucose, T. gambiense was observed to absorb around 30 μ moles of glucose per gram dry weight in 2 minutes (Table 1). This is in close agreement with the data obtained by Christophers and Fulton (1938) and Ryley (1956) for glucose consumption. Ryley's observations were made over an extended time period - 75 min, and glucose removal from the incubation medium was assayed. He found that in 75 minutes, in a CO₂-air (5:95) atmosphere, 18.2 mg dry wt of T. gambiense removed 18.5 μ moles glucose from an incubated medium in which the initial glucose was 15 mM. If it is assumed that uptake was linear with respect to time (the validity of this assumption will be shown below) over the 75 minute interval, this rate is equivalent to 26.6 μ moles glucose per gram dry wt per 2 minutes. The good agreement between Ryley's data and the present observations for glucose uptake is rather exceptional in view of the fact that two entirely different methods were used. Ryley measured glucose consumption for several other haemoflagellates (1956). These data were summarized by von Brand (1966) and shown in Table 11. It is immediately apparent that the African blood-dwelling

Table 11. Aerobic and Anaerobic Glucose Consumption
of Some Trypanosomatidae *

Species	Temperature ° C	Aerobic (μ mole glucose in 1 mg dry tissue in 1 hour)	Anaerobic (μ mole glucose in 1 mg dry tissue in 1 hour)
<u>Trypanosoma cruzi</u> , blood-stream form	37	0.63	1.05
<u>Trypanosoma cruzi</u> , culture form	30	0.24	0.33
<u>Trypanosoma lewisi</u> , blood-stream form	37	0.91	0.81
<u>Strigomonas oncopelti</u> , culture form	30	2.76	3.35
<u>Trypanosoma congolense</u> , blood-stream form	37	1.58	1.77
<u>Trypanosoma vivax</u> , blood-stream form	37	1.37	0.98
<u>Trypanosoma rhodesiense</u> , blood-stream form	37	1.50	1.67
<u>Trypanosoma gambiense</u> , blood-stream form	37	0.80	1.35
<u>Trypanosoma brucei</u> , blood-stream form	37	3.74	1.38
<u>Trypanosoma evansi</u> , blood-stream form	37	4.00	3.10
<u>Trypanosoma equinum</u> , blood-stream form	37	3.22	2.82
<u>Trypanosoma equiperdum</u> , blood-stream form	37	1.71	2.05

*From von Brand, 1966.

trypanosomes have a very high rate of glucose utilization. Although some of the other trypanosomes listed in the table consume glucose rapidly, only the African trypanosomes have an absolute requirement for the presence of a suitable carbohydrate energy substrate without which they quickly perish (Danforth, 1967). As mentioned in MATERIALS AND METHODS, T. gambiense suffers an irreversible loss of motility after only 5 minutes in the absence of glucose or other substrate.

As discussed in the INTRODUCTION, T. gambiense in the presence of oxygen metabolizes glucose almost quantitatively according to the simple relationship:

The stoichiometry of the oxygen to glucose relationship is important. For example, since the relationship of Equation 1 has been well established, it is perfectly satisfactory to measure the metabolic rate of T. gambiense by measuring O_2 consumption. This is not true for some helminth parasites, for instance, which will consume O_2 in its presence but do not require oxygen, at least in large amounts, for normal energy metabolism (von Brand, 1966).

Since in the brucei group at least, O_2 consumption reflects metabolic rate, it is of interest to consider limiting factors which might affect the glucose to pyruvate conversion; the rate

limiting step in glucose metabolism must be considered. The data presented by Ryley (1956) is especially useful in this regard. As calculated previously from Ryley's data, T. gambiense absorbed 26.6 μ moles glucose/g dry wt/2 min. In the same experiment, Ryley reported that 19.42 μ moles O₂ were consumed during the 75 minute period. Making the assumption that O₂ uptake was linear with respect to time, this is equivalent to 28.44 μ moles O₂/g dry wt/2 min. The 28.44 figure for O₂ and 26.6 for glucose are essentially equivalent. Here is experimental verification of Equation 1. What is more important, however, is consideration of the limiting conditions.

The amount of glucose available for metabolism was not limiting in the experiment. The trypanosomes used 18.15 μ moles of the available 60 μ moles in the incubation medium. Thus the glucose concentration was about 10 mM at the end of the experiment. All of the glucose which entered T. gambiense was metabolized (i. e., glucose used equaled O₂ consumed). Reexamination of Figure 1 suggests that both the initial (15 mM) and the final (10 mM) glucose concentrations fall in the saturation area of the glucose transport curve. That is, at 10-15 mM, the rate at which glucose enters T. gambiense is restricted by the transport mechanism, and the glucose site was saturated during the course of the experiment allowing no greater rate of glucose metabolism than was observed. Although glucose available to the trypanosomes in Ryley's experiment was plentiful, its absorption would have been restricted by

the characteristics of the cell membrane. These observations strongly suggest that glucose absorption is the rate-limiting step in the energy metabolism of T. gambiense.

The conclusion that transport is the rate limiting step implies that the other metabolic steps leading to pyruvate formation occur more rapidly than glucose absorption. Although there is at this time insufficient data for all of the enzymatic steps involved, the study conducted by Seed and Baquero (1965) in the characterization of hexokinase from T. gambiense is particularly significant. These workers examined trypanosome hexokinase with special regard to the possibility that the phosphorylation of glucose might be the rate-limiting step. They compared the relative rates of glucose utilization (as determined by O₂ uptake) and glucose phosphorylation in intact cells as well as in cell extracts of Trypanosoma gambiense. They found 1) that rate of glucose utilization (as measured by O₂ consumption) was identical to the amount of glucose phosphorylated by intact cells; 2) that the ability of hexokinase to phosphorylate glucose in cell extracts far exceeded the amount of glucose phosphorylated by intact cells. Seed and Baquero concluded that phosphorylation of glucose, at least, was not the rate-limiting step.

Seed and Baquero also presented other data which is relevant to the discussion now and later. They compared the relative rates of utilization of glucose, mannose and fructose to the ability of hexokinase to phosphorylate these hexoses. It was found that the

difference in rates of utilization of these sugars could not be accounted for by differences in hexokinase activity. In addition, they reasoned that, since the metabolic steps after the formation of fructose-6-phosphate are identical for the various hexoses, the rate of utilization for glucose, mannose and fructose should be identical if one of these steps were rate-limiting. This was not observed, however, and it was suggested that a transport process might be involved. This was particularly indicated by the observation that 2-deoxyglucose inhibited O_2 consumption in intact cells of T. gambiense but not in cell lysates (Seed, et al, 1965).

The present study showing that the rate of glucose entry is restricted at higher substrate concentrations greatly supports the original suggestion by Seed et al (1965) that transport is the rate-limiting step of carbohydrate metabolism in T. gambiense.

This concept can be examined further. The data mentioned above indicate that upon the release to the cell interior from the membrane transport mechanism, hexose is immediately metabolized. Therefore, the amount of free glucose in the cell interior is quite small. That is, in the equation:

$$v = V_{\max} \frac{S_o}{K_m + S_o} + \frac{S_i}{K_m + S_i}$$

the second term $\frac{S_i}{K_m + S_i}$ will be small. In fact, if glucose is

phosphorylated by hexokinase at a much more rapid rate than glucose entry, as has been shown by Seed and Baquero, the $\frac{S_i}{K_m + S_i}$ term can be neglected with the resultant equation:

$$v = V_{\max} \left(\frac{S_o}{S_o + K_m} \right)$$

In any event one would expect that glucose metabolism would be linear with respect to time since S_i will not change with time at a fixed extracellular glucose concentration (Cirillo, 1970). Experimental verification of this expectation comes from the simple observation by Seed and Baquero (1965) that O_2 consumption is linear over 60 minutes.

In connection with this discussion, the subject of active transport should be examined briefly. Most workers now agree that the only criterion for active transport is the accumulation of a substrate against a concentration difference. That is, the cell must have the capacity to maintain the substrate concentration higher on the inner side of the membrane than on the outside. Such a process requires energy, most probably in the form of ATP, and therefore transport systems, where accumulation can be demonstrated, are designated as active.

In view of the arguments presented earlier, the discussion of active transport with respect to T. gambiense glucose transport is academic. Indeed, the ability for T. gambiense to accumulate glucose may be entirely unnecessary, or even superfluous, when the host environment is considered.

The principle location of the vertebrate forms of African trypanosomes is the blood stream. The host for the T.S. strain is presently the white rat, but formerly (see MATERIALS AND METHODS) humans. Although mammalian blood is a rather complex mixture and solution of various components, its composition has been well analyzed and some relevant data (West et al, 1967) will be discussed here. Of special interest to the present problem of glucose metabolism, is the fact that the physiological range of glucose concentration encountered in human blood closely corresponds to the uptake curve presented in Figure 1. Thus the range in concentration of blood glucose is quite large; from 3.5 to 6.5 mM under normal circumstances and even larger under some disease conditions (diabetes, for example). Inspecting the curve in Figure 1, it is evident that blood glucose concentrations in excess of 5 mM will affect very little the carbohydrate metabolism of T. gambiense. Consequently, it appears that under physiological conditions, the glucose site regulates glucose metabolism by preventing excessive glucose from penetrating the cell membrane. Yet the very existence and function of the transport mechanism at these concentrations insures the entry of adequate carbohydrates required for energy metabolism.

Since the physiological blood glucose concentration correlates with the higher parts of the uptake curve in Figure 1, an active transport system for glucose would be superfluous for cell survival. Much of the same argument can be applied to the human

erythrocyte, the membrane of which mediates the transport of glucose without accumulation (Lefevre, 1961).

The uptake curve for ^{14}C -glucose entry into mammalian erythrocytes (Stein, 1967) is remarkably similar to that reported here for T. gambiense. Although the K_m is somewhat lower (0.5 mM), like glucose absorption by T. gambiense, the transport mechanism is essentially saturated at concentrations in excess of 5 mM. Here also, an active transport system would be unnecessary, and indeed, does not exist. It is suggested that the similarity of the glucose-transporting mechanism of erythrocytes and trypanosomes, in terms of similarity of their saturation curves, is no mere accident but rather reflects their common physiological environment.

B. Effect of Temperature. It is not surprising that a decrease in temperature resulted in a decrease in transport rate. The temperature coefficient, Q_{10} , is the ratio of reaction rates at two temperatures ten degrees apart, but a Q_{10} can be calculated given reaction rates at any two temperatures. This assumes that a linear relationship exists; that the Q_{10} between one set of temperatures will be equal to the Q_{10} between two other temperature points. This assumption is not always valid, and therefore the value of Q_{10} calculations, especially in terms of reaction processes, is questionable. This fact is emphasized in the system under study in that Q_{10} values from 1.38 to 2.0 could be calculated depending on the concentration of substrate with which the determination was made. In general,

however, this Q_{10} range of values corresponds to those found for enzymatic reactions. The majority of enzyme catalyzed reactions where such determinations have been made, have Q_{10} values of less than 3 with outer limits being about 1.1 to 5.3 (West, et al, 1967).

C. Absorption of Carbohydrates Other Than Glucose.

The absorption kinetics for fructose, mannose, glycerol, 2-deoxyglucose, and glucosamine were studied (Figures 5 and 6). In all cases the uptake of carbohydrates exhibited saturation kinetics. Such kinetics indicate that the entry of these carbohydrates, like glucose, occurs via a mediated process. Between 1.0 and 10.0 mM, the absorption of glycerol, mannose and 2-deoxyglucose (D-2-D) is more rapid than the uptake of glucose and glucosamine and much more rapid than the uptake of fructose. The calculated K_m 's and V_{max} 's for all of the substrates described in Figures 5 and 6 are shown in Table 3. The V_{max} values are all on the same order of magnitude but there is nearly a 10-fold difference between the K_m 's for 2-deoxyglucose (0.16 mM) and fructose (1.58 mM). It is interesting to note, that the K_m 's for these hexoses is below the physiological concentration range of human blood glucose (3 to 8 mM) and that at these concentrations in Figures 5 and 6, the absorption mechanism appears to be effectively saturated, not only for glucose uptake, but for fructose, mannose, glycerol, 2-deoxyglucose and glucosamine as well.

It is interesting that the non-metabolizable glucose analogue exhibits similar absorption kinetics to those for glucose (Figure 6). Since a rather low concentration of free sugar is maintained within the cell by the hexokinase reaction, as discussed above, we would expect the pattern of D-2-D uptake to be like that for glucose only if D-2-D were also phosphorylated. Seed and Baquero (1965) have demonstrated the phosphorylation of 2-deoxyglucose.

Although 3-O-methylglucose is absorbed by hamster intestine (Crane, 1960), it was found not to be significantly absorbed in 2 minutes by T. gambiense.

The mediated absorption of glucose by Trypanosoma gambiense is not unique. Rather, the entry of hexoses has been found to occur via facilitated mechanisms in practically every cell type studied (Stein, 1967). One of the best known systems is that of the human erythrocyte (see for reviews: Stein, 1967; Lefevre, 1961; Bower, 1957) and is mentioned now since it will be referred to several times below. The erythrocyte system is not only significant because of the amount of information available, but also because it represents a cell type which has an environment identical with that of T. gambiense, namely blood. It is interesting that, like the African blood trypanosomes, the mammalian erythrocyte incompletely oxidizes glucose; unlike the trypanosomes, the major end product is lactic acid.

D. Competition and Inhibition. It has been clearly shown that the entry into Trypanosoma gambiense of several hexoses

occurs via a mediated process, facilitated diffusion. It is of interest to further consider the nature of the transport mechanism, including the number and kinds of carriers located in the trypanosome membrane. The question arises: Do the transported carbohydrates utilize several different carriers specific for each substrate, or is there a single mechanism which facilitates the entry of several sugars? A great deal of information can be obtained, in this respect, by examining the effects of other substrates on the absorption of another substrate. In particular, the demonstration of inhibition with subsequent kinetic analysis can be quite informative. Such data has been obtained for T. gambiense carbohydrate transport, and the inferences possible are important.

Since the discussion which will be presented in this section depends heavily on kinetic data, a brief review of some relevant relationships is in order here (see also INTRODUCTION: A more complete review of carrier transport in kinetic terms has been given by Widdas, 1952; Rosenberg and Wilbrandt, 1957; and Wilbrandt and Rosenberg, 1961). Competitive inhibition will be discussed first and more complex inhibitions will be analyzed as they arise.

Consider the simplified relationship for membrane transport:

where $ES \xrightleftharpoons[k_{-2}]{k_2} ES$ represents membrane translocation of the substrate-carrier complex.

If we assume rate measurement when $S_i = 0$, the formulation:

can be utilized and should result in typical Michaelis-Menton kinetics for enzyme catalyzed reactions.

A competitive inhibitor is a compound which reversibly binds with the transport site, but

When the competitor is itself a substrate, the expression can be given:

only if the same assumptions are made regarding transport of the inhibitor. When these assumptions are not valid, deviation from expected kinetic patterns will occur.

The effect of a transported competitive inhibitor on transport rate of a substrate, assuming the k_2 of D-2 is independent of the k_2 of D-3, is given by the expression:

$$v = \frac{V_{\max}}{1 + \left(\frac{K_m}{S}\right) \cdot \left(1 + \frac{I}{K_i}\right)} \tag{D-4}$$

where I = concentration of inhibitor and K_i = the constant corresponding to K_m , which measures the concentration of the inhibitor required to slow the reaction to half the rate it shows in the absence of the inhibitor.

It can be seen from this equation, that if transport is measured over a concentration range in the presence or absence of a competitive inhibitor (or at two different I concentrations), it should

be observed that ΔI results in a ΔK_m with no appreciable change in V_{max} . Such a relationship was found to exist for T. gambiense with respect to the effects of the various transported carbohydrates upon one another. Each sugar acted as a competitive inhibitor of another when present in pairs; e.g., mannose was found to be a competitive inhibitor of glucose absorption and the reverse was also true.

The information which can be derived from these experiments is somewhat limited compared to the study of inhibition as a function of inhibitor concentration. That is, Lineweaver-Burk plots sometimes indicate whether an inhibitor is a competitor or not, but the effectiveness of inhibition is difficult to extrapolate. The effects of the several carbohydrates upon one another as a function of inhibitor concentrations is indicated in Figures 7 through 12. In Figure 7, the inhibition of uptake velocity is shown, but in Figures 8-12, the percent inhibition (i) is measured, and its relationship to velocity is given by the equation:

$$\underline{i} = \frac{v - v_i}{v} \times 100 \quad (D-5)$$

where v = velocity in the absence of inhibitor
 v_i = velocity in the presence of inhibitor

Determination of i as well as v_i is quite useful in analysis of inhibition.

Figure 8 shows the effects of several carbohydrates on the rate of absorption of ^{14}C -glucose. It can be seen that unlabeled glucose is a very effective inhibitor of glucose uptake. This finding is to be expected according to the hypothesis that glucose entry is a mediated process since the site cannot distinguish between radioactive and non-radioactive molecules. It is important to note two points: 1) glucose in this experiment, acts as its own competitive inhibitor; and 2) by definition, the inhibitor has the same affinity (which may or may not be expressed as K_i ; this point will be discussed below) for the transport site as does the substrate that is being inhibited. These points are highly relevant to other inhibition data to be discussed since the glucose ^{14}C -glucose competition can be used as a standard for the comparison of the action of other competitors.

Figure 8 also demonstrates that mannose inhibition is quite similar to that of cold glucose while glycerol and glucosamine inhibition appears to be significantly different in character.

At this time it is informative to examine these data when plotted on different axes. Thus, Figure 20 is a replot of the data expressed in Figure 8. Here the reciprocal of the percent inhibition (i) is plotted as a function of the reciprocal of inhibitor concentration. As in Figure 8, Figure 20 suggests that mannose and cold glucose behave quite similarly in their effect on ^{14}C -glucose absorption: Notice that they intersect the "X" axis at nearly the same point which is slightly above 0.01 on the $1/i$ axis. This corresponds to the inhibitory pattern at infinitely high

inhibitor concentrations. That is, at infinitely high I, mannose and unlabeled glucose will inhibit the absorption of ^{14}C -glucose nearly 100%. It is important to recognize that this graph implies that mannose and glucose inhibit transport close to but somewhat less than 100%. The reason for the less than 100% inhibition is unknown, but three explanations can be suggested at this time: 1) the observed less than 100% inhibition is an artifact resulting from an obscure source of error; 2) some ^{14}C -glucose enter T. gambiense by simple diffusion rather than a mediated process, and as a result, this amount (which is less than 10% as indicated by the points of intersection of the mannose and glucose lines in Figure 20) cannot be inhibited; and 3) such incomplete inhibition is characteristic of the transport system itself.

The significance of the plots in Figure 20 cannot be overemphasized. These data suggest molecules of mannose and unlabeled glucose behave similarly as competitors, and it can be inferred qualitatively that mannose and glucose have similar affinities for the glucose site. Compare now the effect of glucosamine on glucose absorption as shown in Figure 8 and 20. Figure 8 demonstrates that, in the concentration range studied, glucosamine is not as effective an inhibitor as mannose. Figure 20 shows that at infinite glucosamine concentrations, the absorption of ^{14}C -glucose will be inhibited only about 50%. How can this be explained in view that glucosamine was found to competitively inhibit glucose absorption? The fact that mannose inhibits glucose uptake some 90% dictates that this 50%

discrepancy cannot be attributed to glucose diffusion as suggested above.

Returning to the earlier discussion concerning the kinetics of inhibition, we see that the observed kinetics for glucosamine inhibition cannot be explained by the simple kinetic relationships already mentioned, e.g., the observed inhibition by glucosamine does not conform to the relationship described in Equation D-4. That is, some or all of the assumptions made to make the formulations in D-1, D-2, D-3 and D-4 were somehow not justified. This can be demonstrated further by plotting $1/v$ as a function of inhibitor concentration. The same data expressed in Figures 8 and 20 are replotted in Figure 21. It is immediately obvious that instead of a linear relationship which is predicted by Equation D-4, the plots for each inhibitor, including unlabeled glucose, are not linear. Clearly the transport system is more complex with respect to inhibitors than originally envisioned.

Webb (1963) has described such inhibitory patterns for enzymes and has applied the term "partially competitive" to inhibitions where curved lines result from $1/v$ vs. I plots and lines intersecting the "X" axis at points greater than 0.01 in $1/i$ vs. $1/I$ plots. Therefore, it can be said that glucose transport is partially competitively inhibited by glucosamine as evidenced by Figures 20 and 21.

The same arguments can be applied to the effects of glycerol on glucose uptake. Figures 20 and 21 indicate that glycerol inhibition is also partially rather than wholly competitive. We see, however, that glycerol is a much more potent inhibitor than is glucosamine.

It is significant that when plotted as in Figure 21, it would appear that the inhibition of glucose transport is partially competitively inhibited by unlabeled glucose.

Examination of Figures 22 and 23 reveals the effects of several competitors on mannose transport. Consider first the effect of glucose. Glucose appears to inhibit mannose uptake nearly 95% (Figure 22) in the same way that mannose inhibited glucose uptake (Figure 20). The effect of 2-deoxyglucose is quite similar, and it would appear that the effect of glucose and 2-deoxyglucose and mannose uptake is identical with the exception that the slopes of the lines in Figures 22 and 23 are different. That these slopes differ suggests that the two sugars, by nature of their structural differences, have different rate constants (different affinities) with respect to the transport site. The inhibition pattern for glycerol is also shown in Figures 22 and 23. It is apparent that glycerol inhibition of mannose uptake is similar to that for glucose uptake; the inhibition is partially competitive.

At this point some conclusions may be drawn with respect to glucose, mannose and glycerol transport. It has been

shown that glucose and mannose exhibit mutual inhibition of transport. Since both hexoses are themselves transported, it can only be concluded that these hexoses share the same absorption mechanism which shall be referred to hereafter as the glucose site. The glucose site, then, facilitates the entry of mannose and glucose. These hexoses differ from each other only in configuration of the hydroxyl group about carbon number 2. The great similarity of glucose and mannose in terms of inhibition and uptake kinetics suggests that the glucose site cannot distinguish these sugars to any great degree.

What can be said about glycerol? This alcohol is a partially competitive inhibitor of the glucose site (Figures 20, 21, 22 and 23). Still glycerol exhibits mutual competitive inhibition for mannose and glucose transport. Therefore, it can only be concluded that glycerol also shares the glucose site.

It is significant to note that Figure 24 suggests that mannose and glucose act as partial competitors of glycerol absorption. Therefore pairs of glycerol and mannose or glycerol and glucose behave as partially competitive inhibitors of one another. In view of these data, the hypothesis is suggested here that with respect to a transport system, such as the one found in T. gambiense, two compounds will exhibit partially competitive inhibition of one another if their entry is mediated by a common mechanism. It is also suggested that this behavior is more the rule than the exception under certain conditions of substrate, inhibitor, and measurement conditions.

For example consider the effects of glucosamine, glucose and fructose upon one another. It appears from Figure 8 that glucosamine is not as effective an inhibitor of glucose transport as unlabeled glucose. This is confirmed in Figures 20 and 21 which also demonstrate that the nature of the inhibition is partially competitive. These data also indicate that, qualitatively at least, as evidenced by differences in the slope of the lines in $1/i$ vs. $1/I$ or $1/v$ vs. I plots, the affinities of glucose and glucosamine for the glucose site differ.

The effect of fructose upon glucose and glucosamine uptake is shown in Figure 13. It is clear that fructose is a very weak inhibitor of glucose uptake but a much stronger inhibitor of glucosamine uptake. Although not apparent in Figure 25 (these plots do not always detect partially competitive inhibitors), fructose was found to be a partially competitive inhibitor of glucosamine uptake. It is also clear that glucose is a partially competitive inhibitor of glucosamine uptake (Figure 25).

From Figure 26 and 27 it is apparent that all of the competitors tested exhibited partially competitive inhibition of fructose transport.

Although not shown here because of the very weak inhibition exerted by this ketose, fructose was found to be a partially competitive inhibitor of glucose uptake. It can be concluded from all these data that glucose, glucosamine and fructose act as partially

competitive inhibitors of one another when tested in pairs.

The effects of some other substrates upon one another are shown also in Figures 20-27. Further plotting of substrate interactions in all possible combinations of pairs seems somewhat redundant at this time, but such information can be obtained by replotting all of the data expressed in Figures 8-13. Suffice it to say then, that most of the inhibitions exhibited for the glucose site are of the partially competitive type. It would appear also that the greater the similarity of substrates in "affinities" for the glucose site, the less the degree of partially competitive inhibition.

It is important to interject that relative rates of simple absorption may have little meaning with respect to inhibition kinetics. That is, it is evident from the uptake kinetics of the various ^{14}C -substrates that the ability to act as an inhibitor cannot be equated with the ability to be transported as determined by the kinetic parameter, K_m . This is not surprising if the K_i of an inhibitor is not equal to k_{-1}/k_1 (the dissociation constant for the EI complex) but rather is $(k_{-1} \cdot k_2)/k_1$. Likewise, unless k_2 is very much greater than k_{-1} (an assumption that cannot be made), K_m does not equal K_s and K_m is not a measure of the affinity of a substrate for the site.

At this time reexamination of Figures 14 and 14-A is informative. It is clear that glucose, mannose, glycerol, glucosamine and fructose (see also Figure 13) exhibit mutually competitive inhibition of one another. It is equally clear that fructose inhibition of glucose

is so small at the lower I/S ratios tested, that it could be suggested that this inhibition is not significant. If this conclusion is made, the simplest way for which the data can be accounted is the supposition of a two site model. One site, the glucose site, mediates the uptake of glucose, mannose, glycerol and 2-deoxyglucose while not transporting fructose (from the observation that fructose does not greatly reduce glucose absorption). The second site, the fructose site, mediates the transport of fructose. The observation that glucose inhibits fructose absorption quite effectively can be accounted for if glucose reacts with the fructose site while not being transported by it. Glucosamine reacts with the glucose site but is not transported by it; rather, it is absorbed via the fructose site. This model will be referred to as the two site hypothesis which was originally suggested by Southworth and Read (1969).

An alternative interpretation of the observed inhibition kinetics is perhaps more tenable. The proposed model assumes the existence of only one locus, the glucose site, which mediates the transport of glucose, mannose, glycerol and 2-deoxyglucose as well as the transport of glucosamine and fructose since inhibition of substrate transport is significant at higher I/S ratios (Figure 13). This model can account for all of the observed inhibition kinetics including the small but significant inhibition of glucose absorption by fructose if it is assumed (as the experimental data indicate) that the transported carbohydrates differ in their "affinities" for the glucose site.

Consider briefly the significance of substrate "affinity".

It should be noted (see also INTRODUCTION) that the K_m can be used as a measure for substrate affinity in the formulation:

$$K_s = \frac{k_{-1}}{k_1} \quad \text{and} \quad K_m = \frac{k_{-1} k_2}{k_1}$$

if and only if k_2 is rate limiting: i.e., $k_2 \ll k_{-1}$. The same arguments apply to an inhibitor where $K_i = K_s$ (of inhibition) only if the k_2 for the EI complex is rate limiting. Let us assume that in a model system where a competitor is being tested on an inhibitor, the K_m and the K_i for the substrate and inhibitor do represent their dissociation constants (K_s 's) and therefore their affinities for a site. Using the relationships of Equation D-4, we can examine how substrates with greatly different affinities toward a common site can influence each other's kinetics.

Rearranging Equation D-4, we get the relationship:

$$v = \frac{V_{\max}}{(1) + \frac{K_m}{S} + \frac{K_m I}{S K_i}} \quad (D-6)$$

[Assume that S remains constant and that $S = K_m$. It is clear that not only as I increases, v decreases, but also this decrease

in I can be greatly affected by K_m and K_i . For example, if $K_m \gg K_i$, the third term of the denominator will be quite large compared to the others and a small increase in I will result in a large decrease in velocity. Compare this to the situation in which $K_i \gg K_m$; in this case the third term of the denominator will be quite small compared to the rest of the denominator and a large increase in I will not greatly decrease velocity.

It can be inferred from these observations that two situations can arise when a pair of substrates which differ largely in terms of affinities for a site, are tested for mutual inhibition under fixed conditions:

1) The substrate with the greatest affinity will greatly inhibit the transport of the substrate with the lesser affinity. In this case $K_m \ll K_i$; 2) if the second substrate of lesser affinity is tested as an inhibitor of the first substrate under the same S and I conditions, in this case $K_i \gg K_m$, comparatively little inhibition will be exhibited and may even be entirely masked depending on the concentrations and ratios of I and S.

These relationships are merely a function of the kinetic constants involved and have been discussed with respect to enzymes (Webb, 1963) and carriers (Lefevre, 1961).

The data suggest that the inhibition kinetics exhibited by glucose and fructose upon one another are a result of widely different affinities toward the T. gambiense transport system. The fact that their apparent K_m 's are not widely divergent does not contradict this hypothesis since the molecular significance of K_m with respect to sugar transport in this parasite is unknown and the K_m is probably not a true measure of affinity for the site. That is, it seems likely that K_m

represents many kinetic constants, and the assumption that $K_m = K_s$ is probably not valid.

It is interesting to note at this time that a similar glucose-fructose relationship was observed by Lefevre (1954) in the human erythrocyte. While glucose greatly inhibited fructose absorption, the reverse could not be shown. It was observed by Lefevre that, with respect to affinity for the glucose site, glucose $\gg$ fructose.

Perhaps more convincing evidence that glucose and fructose share a common transport locus is the interaction of these two sugars with the amino sugar, glucosamine. Glucosamine exhibits mutual competitive inhibition with both fructose and glucose (Figure 14-A). Since glucose and glucosamine are mutually inhibitory, it can be inferred that they share the same site. Since fructose and glucosamine are mutually inhibitory, it can be inferred that they also share the same transport site. Therefore it would be likely that glucose and fructose share the same transport mechanism.

The relative interaction of these three sugars with respect to degrees of inhibition of one another support the view that fructose is a poor inhibitor of glucose because it has a low (relative to glucose) affinity for the transport site. As was pointed out in RESULTS, the data indicate that glucose $\gg$ glucosamine $\gg$ fructose in order of their ability to exert inhibition of one another. These results are consistent with the one site hypothesis if this ranking results in a similar ranking in affinity of these three sugars for the glucose site. On the other

hand, the relative interaction of glucose, glucosamine, and fructose would be very difficult to explain in terms of a two site model.

The significance of partial competitive inhibition will be considered now. Let us consider the more general relationship for facilitated diffusion as expressed by Stein (1967):

The kinetics of D-7 are much more complex in the presence of a competitive inhibitor which is itself transported. That is, the competition will affect the kinetic constant, K_m , at several levels since it is likely, for example, that k_2ES will not be equal to k_2EI .

It can be shown that for competitive inhibition:

$$\underline{i} = \frac{I}{I + K_i (1 + S/K_m)} \cdot 100 \quad (D-8)$$

where $\underline{i}$ = percent inhibition. It can be seen, that as I becomes very large, $\underline{i}$ approaches 100. With partially competitive inhibition of the type* observed for T. gambiense it is apparent that $\underline{i} \nrightarrow 100$ even at infinite inhibitor concentrations (see, for example, the effect of glycerol on mannose in Figure 22). It is apparent then that $\underline{i}$ is also dependent on another term (not expressed in D-8) which will approach a constant, which we shall designate γ , as I approaches infinity. Therefore, at $I \approx \infty$

$$\underline{i} \approx \gamma \cdot (100) \quad (D-10)$$

where $\gamma < 1$.

* The demonstration of partially competitive inhibition by $1/v$ vs. I plots or $1/i$ vs. $1/I$ plots says nothing about the molecular events involved. In fact partially competitive inhibition can arise as a result of a variety of situations or assumptions - whichever the case. As pointed out by Webb (1963) partially competitive inhibition means that the inhibition is still a competition, but that the degree of inhibition is less than that of a completely competitive system. What this means with respect to sugar transport is that the transported inhibitor is not able (even at infinite I) to completely eliminate the absorption of another sugar.

The data indicate that transport does not exhibit kinetics consistent with the hypothesis that the inhibitor and the substrate act independently when present simultaneously. That is to say:

$$E_t \neq E + ES + EI \quad (D-10)$$

where E_t = total carrier
E = unbound carrier
ES = carrier substrate complex
ES = carrier inhibitor complex

Since the kinetics do not conform to this supposition, the total carrier involved is not accounted for in the conservation equation D-10. It is clear that whatever the unknown carrier component, it must depend on I and S, and must influence the binding of S and vice versa.

Webb (1963) has discussed partially competitive inhibition under special conditions of an inhibitor reacting with an apoenzyme but these formalisms are not applicable to transport. In general, it is not difficult to derive rate equations which can describe partially competitive inhibition. Such propositions, unfortunately, are premature for it is obvious that the exact molecular events involved are complex. It will be of great interest to experimentally determine if the hypothesis proposed above that two transported substrates will exert partially competitive inhibition on one another will be applicable to transport phenomena in general, or if it is only a characteristic of certain transport systems.

E. Specificity of the Glucose Site. The data described in the present study support the hypothesis that a single mechanism facilitates the transport of glucose, fructose, mannose, 2-deoxyglucose, glucosamine and glycerol into T. gambiense. These data also suggest that the glucose site is somewhat non-specific in that it reacts with several carbohydrates other than glucose. That the specificity of the glucose site is much greater, however, is evidenced by Table 6. Thus a large number of carbohydrates were tested as inhibitors of glucose and fructose transport at fixed I/S ratios (10:1) and were found not to affect glucose or fructose uptake to a detectable extent. This is not to say, of course, that these substances might not produce inhibition under different experimental conditions.

Several glycerol analogues were tested as inhibitors of glucose or fructose transport. Both 1,3-propanediol (2-deoxyglycerol) and 1,2-propanediol resemble the glycerol molecule very closely, yet these did not affect glucose transport. Also ethylene glycol, 1-propanol and propionic acid were ineffective. In contrast, ethylene glycol, 1,3 and 1,2 propanediol are inhibitors of the glycerol transport system of human erythrocytes (Christensen, 1962).

With respect to glucose analogues, it would appear from Table 6 that the glucose site is more specific than originally suspected. For example several hexoses which differ from glucose in configuration or substitution only about a single carbon atom are not effective inhibitors. For example, D-galactose, which differs from glucose only

in configuration of the hydroxyl group at carbon-4, did not inhibit transport. Similarly, several carbon-1 substituted glucose analogues were not inhibitory. Thus gluconic acid, α -methyl glucoside, D-mannitol, D-sorbitol, and phenylglucopyranoside did not inhibit glucose or fructose transport. Likewise substitutions at carbon-6 (gluconic acid) and at carbon-3 (3-O-methylglucose) were without effect. The failure of 3-O-methylglucose to be transported corresponds to its inability to inhibit glucose uptake in Table 6. The specificity of carbon-3 is further emphasized by the fact that D-allose, which varies from the structure of glucose only in configuration of the hydroxyl group at carbon-3, did not inhibit glucose uptake.

It is significant that no inhibition was observed with L-sorbose which is identical to the structure of D-fructose with the exception of configuration of the hydroxyl group at carbon number 5. Equally noninhibitory was D-tagatose which differs from D-fructose only at carbon-4.

At this point it should be quite apparent that the hexoses which do utilize the glucose site share a common characteristic: each is a carbon-2 epimer of glucose. Thus the structure of mannose is identical to glucose except for configuration of the hydroxyl group at carbon-2. It is clear that elimination of the hydroxyl group does not greatly influence transport since the absorption of 2-deoxyglucose (which is also 2-deoxymannose) is mediated by the glucose site. Significantly the ketone group of fructose is at the number 2 carbon.

It is evident that limited substitution at carbon-2 will still permit reaction with the transport mechanism but there is a definite limit. This is shown by the fact that substitution of an amino group at carbon-2 (glucosamine) does not greatly reduce transport. On the other hand, N-acetyl-glucosamine was an inhibitor of glucose and fructose transport; this hexose was not itself absorbed via a mediated process (Figure 19). This suggests that, although specificity at the number 2 carbon is not great, it has finite limits.

That glucose and glycerol might share the same transport locus was originally suggested by von Brand et al (1967). Their suggestion has been confirmed by the data presented here. Thus the T. gambiense system is in contrast to the human erythrocyte where glycerol absorption is facilitated by an entirely separate mechanism than is the absorption of glucose (Stein, 1967).

As has been discussed, with respect to the transported hexoses, specificity at carbon-2 is not great. With this exception the transported hexoses (except for fructose by nature of its furanoside or pyranoside structure in solution) are identical. The relationship between the alcohol (glycerol) and glucose is not so obvious. In this respect it is helpful to compare their configurations using molecular models. Such comparisons reveal that, of the conformational possibilities for glycerol and glucose, the most favorable comparisons are between the glycerol molecule and glucose carbons-3, 4 and 5 or between glycerol and glucose carbons-4, 5 and 6. The greatest dissimilarity occurs

when glycerol is compared to carbon-1, 2 and 3 of glucose, and this is corroborated since carbon-2 does not appear to be important to the glucose transport mechanism.

When the glycerol molecule is in the same conformation corresponding to carbons-4, 5 and 6 of the glucose molecule the relative positions of the hydroxyl groups of the glycerol molecule are energetically most favorable. The same can be said concerning carbons position 3, 4 and 5 of the glucose molecule. When the glycerol molecule is orientated such as to compare with the carbons 4, 5 and 6, only one hydroxyl group at the number 2 glycerol position and one hydrogen at the number 1 glycerol position do not conform to the structure of glucose. When the glycerol molecule is orientated such as to compare with carbons 3, 4 and 5, the groups at the number 2 glycerol position conform to the number 5 carbon position, while at one of the number 1 glycerol carbons, the hydrogen group of the glycerol does not conform to its analogous position, and the number 3 glycerol position does not compare with respect to one hydrogen group and one hydroxyl group. On this basis, it can be inferred that the greatest similarity between the glycerol molecule and the glucose molecule lies in comparing glycerol to carbons 4, 5 and 6 of the glucose molecule.

It has become apparent that transport systems so far investigated have many similarities and as many dissimilarities. An informative comparison of the T. gambiense transport system with other systems with respect to number and kind as well as specificity

of substrates transported would be an enormous and tedious task. Let it suffice to say that the sugar transport system described here for T. gambiense does not appear to be unusual in its specificity requirements.

F. Effect of Metabolic Poisons and Other Inhibitors.

The development of biochemistry has often depended on the use of organic and inorganic poisons. The more specific the action of an inhibitor, the better it can be utilized to elucidate a particular mechanism. Unfortunately, many of the metabolic inhibitors commonly used in biochemical analysis are quite nonspecific at the molecular level, and this fact has often been overlooked. Nor is it always determined at what level an inhibitor exerts its action. For example cellular compartmentation is of paramount importance since some cellular membranes may be permeable to the inhibitor while others are not. That these considerations are quite important is indicated in the data obtained for T. gambiense.

Some well known metabolic inhibitors were tested for their effects on glucose transport in T. gambiense (Table 7). Several of these were found to be ineffective. Such negative results must be interpreted with caution. It must be noted, for example, that 2,4-dinitrophenol was not inhibitory in 2 minute incubations. Yet it could be argued that if the membrane of T. gambiense were not greatly permeable to this compound, 2 minute incubations are too short to allow penetration of significant quantities of dinitrophenol.

It is now well established in a few systems such as the human erythrocyte, that ouabain selectively inhibits the $\text{Na}^+ - \text{K}^+ - \text{Mg}^{++}$ activated ATPase and will, in turn, inhibit the active extrusion of sodium ions (Whittam & Wheeler, 1970). Since ouabain appears to exert its effect at the cell exterior in these systems, 2 minute incubation should be sufficient to demonstrate inhibition. Crane (1965) has described a model which couples sodium transport with glucose accumulation in the mammalian intestine. The experimental evidence in support of the co-transport of Na^+ and sugar is impressive and its general applicability to other systems (i. e. , amino acid accumulation in ascites cells) has been indicated (Stein, 1967). More specifically, Crane's model links the "sodium pump" with the active accumulation of sugar and interference with the active transport of sodium will affect glucose accumulation. Thus ouabain, acting on the sodium pump via the $\text{Na}^+ - \text{K}^+ -$ activated ATPase, prevents accumulation of glucose by hamster intestinal mucosa (Stein, 1967). In the presence of ouabain, the glucose transport system exhibits properties of facilitated diffusion - sugar will move with a concentration gradient but not against it.

It is significant therefore that not only did ouabain not affect transport in T. gambiense, but also the replacement of Na^+ with tris ions (Section H of RESULTS) did not influence the rate of sugar transport.

Iodoacetic acid and HgCl_2 proved to be strong inhibitors of glucose transport. This finding is not particularly meaningful since

it is well known that these compounds are potent inhibitors of accessible sulfhydryl groups of proteins. However, such inhibition is significant in consideration of other data. Von Brand et al (1950) and von Brand et al (1953) tested the effects of several arsenicals, fluoride, and sulfhydryl inhibitors on the uptake of oxygen by T. gambiense. They found that iodoacetate, fluoride and sodium arsenite, among others, greatly reduced O₂ consumption by the parasites. It is clear from Table 7 that these compounds do not exert this action at the same level. Thus it appears that arsenite and fluoride inhibit glucose metabolism subsequent to phosphorylation while iodoacetate acts at the transport level. Such information may be quite useful in future metabolic studies.

That phlorizin was found to be an inhibitor of glucose transport is certainly not unique. Although its inhibitory effect is not identical for various cell types, phlorizin has been found to be one of the most successful inhibitors of glucose transport (Stein, 1967). Although the data presented here gives no indication of the type of inhibition exerted, its action is competitive with respect to sugar transport by hamster intestine (Alvarado and Crane, 1962).

G. Significance of Glucose Absorption in Trypanosoma gambiense and the Relation to Other Organisms. That the operation of the glucose carrier functions in a physiological range has been discussed in Section A. It has also been pointed out that there is no evidence to indicate the existence of an active transport mechanism in

T. gambiense for the accumulation of sugar, and, indeed, there seems to be no necessity for one.

For other organisms, however, the availability and concentrations of metabolizable substrates fluctuates greatly and is probably limiting. In such cases an additional regulatory device for the uptake of substrates is needed to maintain homeostasis. The ability to accumulate these substrates against a concentration gradient would be such a device. Although a cause and effect relationship cannot be a certainty in a complex system, it is significant that mammalian intestine can accumulate glucose. It is intuitively obvious that such a mechanism is necessary if glucose concentration in the lumen is less than in the circulatory system, just as such a mechanism is necessary for kidney reabsorption. It is interesting and perhaps more than coincidence that the mammalian tapeworm, Hymenolepis can accumulate glucose (Phifer, 1959, 1960).

Many systems have been studied with respect to active transport of organic compounds. In most cases, a physiological need for active transport can be ascribed to the system in which it is found. In contrast, some cells would not possess a physiological advantage with an active transport mechanism. In these cases, the red cell for example, such mechanisms do not exist.

There have been a few studies on the nature of carbohydrate transport in hemoflagellates. Warren (1963) investigated the absorption of ^{14}C -labeled galactose and fructose by the culture form of T. cruzi,

the agent of South American trypanosomiasis, and demonstrated that this flagellate absorbs fructose more rapidly than galactose and that the uptake of these sugars could be greatly inhibited by glucose. From the data obtained, Warren suggested that the T. cruzi culture organisms could accumulate fructose but not galactose against a concentration difference. Galactose entry appeared to occur by facilitated diffusion. It is important to interject that hemoflagellates grown in culture presumably correspond to that stage of the life cycle parasitizing the insect gut. In this case, an active transport system for the acquisition of metabolizable substrates may be of physiological advantage for the parasite living in such an environment. In support of the suggestion that the insect gut environment requires special adaptations (namely active transport) are the studies by Min (1965, 1966) on another hemoflagellate, Crithidia luciliae. Min demonstrated that this parasite could accumulate some sugars against a concentration difference. Since C. luciliae is exclusively a gut parasite, he suggested that such a mechanism might be an adaptation to conditions of the habitat of this parasite; the ability to accumulate sugars would be of physiological advantage when glucose concentration of the gut remained low for extended time periods.

Glucose transport has been studied to some extent in the blood stream form of the rat trypanosome, T. lewisi by Sanchez & Read (1968). It is significant that, like T. gambiense, the saturation of glucose uptake occurred at physiological blood glucose concentrations. Although the evidence does not rule out the existence of an active transport

system, it appears that the physiological entry of glucose into T. lewisi, like that in T. gambiense, occurs via facilitated diffusion.

While the evidence is limited to only four organisms, the trend within the hemoflagellates is clear: 1) blood parasites utilize a facilitated diffusion mechanism for glucose entry at physiological concentrations; 2) insect gut parasites possess, and presumably utilize, an active transport mechanism for sugar entry. It was suggested at the end of Section A of this discussion and is emphasized again here that the mechanism and character of carbohydrate transport should reflect the environment in which the organism or cell type must exist. The already mentioned great similarity between glucose entry in human erythrocytes and the blood trypanosomes bears this out. Other parasitisms which have been studied in this respect conform to such a pattern. The similarity of mechanism for sugar entry for the mammalian intestine and the intestinal tapeworm has been mentioned.

Further comments on the study of Sanchez and Read (1969) on carbohydrate transport in T. lewisi are in order at this time. It was suggested in Section D of this discussion that the phenomenon of partially competitive inhibition exerted by one transported substrate upon another was symptomatic of complex molecular events involved in the translocation process, and that it perhaps was a characteristic applicable to facilitated diffusion mechanisms in general. It is noteworthy that Sanchez and Read found that the nature of inhibition by analogues of ^{14}C -glucose and ^{14}C -fructose transport was partially competitive.

These authors suggested this fact to mean the existence of more than one site for hexose transfer but in view of the evidence obtained with T. gambiense and the considerations discussed in Section D, it is possible to interpret their data in terms of a single transport site the affinity for which the several carbohydrates they tested were different.

APPENDIX A

Composition of KRT Buffer:

120.0 mM NaCl
4.8 mM KCl
2.6 mM CaCl₂
1.2 mM MgSO₄
25.0 mM tris (hydroxymethyl) amino methane

pH adjusted to 7.4 with NaOH

BIBLIOGRAPHY

- Allain, D. S. 1964. Evaluation of the viability and pathogenicity of hemoflagellates after freezing and storage. *J. Parasit.* 50:604-607.
- Alvardo, F. and R. K. Crane. 1962. Phlorizin as a comparative inhibitor of the active transport of sugars by hamster small intestine in vitro. *Biochim. Biophys. Acta.* 56:170-172.
- Bower, B. F. 1967. The Kinetics of the penetration of non-electrolytes into the mammalian erythrocyte. *Intern. Rev. Cytol.* 6:469-511.
- Browning, C. M. and R. Glubransen. 1935. Variation in chemotherapeutic susceptibility associated with change in virulence of a strain of Trypanosoma brucei. *J. Hyg.* 35:180.
- Bruce, D. 1911. The morphology of Trypanosoma gambiense. *Sleeping Sickness Bulletin B.* 327-332.
- Christensen, H. N. 1962. Biological transport. W. A. Benjamin, Inc. New York.
- Christophers, S. R. and J. D. Fulton. 1938. Observations of the respiratory metabolism of malaria parasites and trypanosomes. *Ann. Trop. Med. Parasitol.* 32:43-75.
- Cirillo, V. P. 1970. The role of membrane carriers in the regulation of the free glucose pool in metabolizing cells. *J. Protozool.* 17:178-181.
- Coggeshall, L. T. 1939. Preservation of viable malaria parasites in the frozen state. *Proc. Soc. Exptl. Biol. Med.* 42:499-501.
- Crane, R. K. 1960. Intestinal absorption of sugars. *Physiol. Rev.* 40:789-825.
- Crane, R. K. 1964. Uphill outflow of sugar from intestinal epithelial cells induced by reversal of the Na^+ gradient: Its significance for the mechanism of Na^+ -dependent active transport. *Biochem. Biophys. Res. Commun.* 17:481-485.

- Crane, R. K. 1965. Na^+ -dependent transport in the intestine and other animal tissues. *Federation Proc.* 24:1000-1006.
- Cunningham, M. P., W. H. R. Lumsden, K. Van Hoeve, and W. A. F. Webber. 1963. The survival of trypanosomes of the Trypanosoma brucei sub-group at low temperatures. *Biochem. J.* 89:73-74.
- Danforth, W. F. 1967. Respiratory metabolism in Chen, T., Research in Protozoology, Pergamon Press, New York. 1:201-306.
- Fulton, J. D. 1969. In Research in Protozoology. Vol. 3. Pergamon Press, New York.
- Fulton, J. D. and Audrey V. Smith. Preservation of Entamoeba histolytica at -79 C in the presence of glycerol. *Ann. Trop. Med. Parasitol.* 47:240-246.
- Glynn, I. M. and M. B. Chir. 1968. Membrane adenosine triphosphatase and cation transport. *Brit. Med. Bull.* 24:165-169.
- Goble, D. G., B. Ferrell and A. R. Stieglitz. 1959. The virulence and drug susceptibility of certain strains of trypanosomes of the brucei - evansi group maintained by syringe passage. *Ann. Trop. Med. Parasitol.* 53:189.
- Lefevre, P. G. 1961. Sugar transport in the red blood cell: Structure-activity relationships in substrates and antagonists. *Pharmacol. Rev.* 13:39-70.
- Lefevre, P. G. and R. L. Davies. 1954. Active transport into the human erythrocyte: Evidence from comparative kinetics and competition among the monosaccharides. *J. Gen. Physiol.* 34:515-524.
- Levine, N. D. and W. C. Marguardt. 1955. The effect of glycerol and related compounds in survival of Tritrichomonas foetus at freezing temperatures. *J. Protozool.* 2:100-107.
- Lumsden, W. H. R. 1967. Trends in research on the immunology of trypanosomiasis. *Bull. Wld. Hlth. Org.* 37:167-175.

- Manwell, R. D. 1943. The low-temperature freezing of malaria parasites. *Am. J. Trop. Med.* 23:123-131.
- Min, H. S. 1965. Studies on the transport of carbohydrate in Crithidia luciliae. *Jour. Cell Comp. Physiol.* 65:243-249.
- Min, H. S. 1966. Effects of inhibitor, competitors and temperature of transport of carbohydrate in Crithidia luciliae. *Jour. Cell. Physiol.* 68:237-240.
- Ormerod, W. E. 1967. Taxonomy of the sleeping sickness trypanosomes. *Journ. Parasit.* 53:824-830.
- Phifer, K. 1960a. Permeation and membrane transport in animal parasites: Further observations on the uptake of glucose by Hymenolepis diminuta. *J. Parasit.* 46:137-144.
- Phifer, K. 1960b. Permeation and membrane transport in animal parasites: On the mechanism of glucose uptake by Hymenolepis diminuta. *Journ. Parasit.* 46:145-153.
- Polge, C. and M. A. Soltys. 1957. Preservation of trypanosomes in the frozen state. *Trans. Roy. Soc. Trop. Med. Hyg.* 51:519-526.
- Rosenberg, T. and W. Wilbrandt. 1955. The kinetics of membrane transport involving chemical reactions. *Exptl. Cell Res.* 9:49-67.
- Ryley, J. F. 1956. Studies on the metabolism of the Protozoa 7. Comparative carbohydrate metabolism of eleven species of trypanosomes. *Biochem. J.* 62:215-222.
- Sanchez, Gilbert and C. P. Read. 1969. Carbohydrate transport in Trypanosoma lewisi. *Comp. Biochem. Physiol.* 28:931-937.
- Saunders, G. M. and V. Scott. 1947. Preservation of Plasmodium vivax by freezing. *Science.* 106:300-301.
- Seed, J. R. and M. A. Baquero. 1965. The characterization of hexokinase from Trypanosoma rhodesiense and Trypanosoma gambiense. *J. Protozool.* 12:427-432.

- Seed, J. R. and M. A. Baquero and J. F. Duda. 1965. Inhibition of hexose and glycerol utilization by 2-deoxy-D-glucose in Trypanosoma gambiense and Trypanosoma rhodesiense. Exp. Parasit. 16:363-368.
- Southworth, G. C. and C. P. Read. 1969. Carbohydrate transport in Trypanosoma gambiense. J. Protozool. 16:720-723.
- Stein, W. D. 1967. The Movement of Molecules Across Cell Membranes. Academic Press, New York.
- Taliaferro, W. H. 1941. In Protozoa in Biological Research. Calkins and Summers, Ed. Columbia University Press, New York.
- von Brand, T. 1966. Biochemistry of Parasites. Academic Press, New York.
- von Brand, T., E. J. Tobie and H. Higgins. 1967. Hexose and glycerol absorption by some trypanosomatidae. J. Protozool. 14:8-14.
- von Brand, T., E. J. Tobie and B. Mehlman. 1950. The influence of some sulfhydryl inhibitors and of fluoroacetate on the oxygen consumption of some trypanosomes. Jour. Cell. Comp. Physiol. 35:273-300.
- von Brand, T., E. J. Tobie, B. Mehlman and E. C. Weinbach. 1953. Observations of the metabolism of normal and arsenic-resistant Trypanosoma gambiense. Jour. Cell. Comp. Physiol. 41:1.
- Warren, L. G. 1963. Metabolismo del Schizotrypanum cruzi Chagas. III. Evidencia en favor de un mecanismo de transporte activo de la galactose y la fructosa. Acta. Cent. Venezolana Suppl. 1:127-134.
- Webb, J. L. 1963. Enzyme and Metabolic Inhibitors. Vol. 1. Academic Press, New York.
- West, E. S., W. R. Todd, H. S. Mason and J. T. Van Brugyn. 1966. Textbook of Biochemistry. 4th Ed. Macmillan, New York.
- Whittam, R. and K. P. Wheeler. 1970. Transport across cell membranes. In Annual Review of Physiology. :21-60.

- Widdas, W. F. 1952. Inability of diffusion to account for placental glucose transfer in the sheep and consideration of the kinetics of a possible carrier transfer. J. Physiol. (London). 118:23-39.
- Wilbrandt, W. and T. Rosenberg. 1961. The concept of carrier transport and its corollaries in pharmacology. Pharmacol. Rev. 13:109-183.

Figure 1.

The uptake of ^{14}C -glucose by *T. gambiense* at 37 C
as a function of glucose concentration.

The incubation time was 2 minutes.

Figure 2.

Linear Regression Analysis of the Data Expressed
in Figure 1.

K_m for glucose transport = 1.35 millimolar

V_{max} = 47.9 μ moles/g dry wt/2 min.

Figure 3.

Effect of incubation time on the absorption
of ^{14}C -glucose at 37 C.

Figure 4.

Effect of temperature on the absorption
of ^{14}C -glucose and ^{14}C -fructose in 2 minute
incubations.

Figure 5.

Absorption of ^{14}C -glucose and ^{14}C -glucosamine at 37 C.

Figure 6.

Uptake of ^{14}C -glycerol, mannose, fructose and
2-deoxyglucose (D-2-D) by T. gambiense at 37 C.

Figure 7.

Inhibition of 2.5 mM ^{14}C -glucose absorption
by 2-deoxyglucose.

Figure 8.

Inhibition of 2.5 mM ^{14}C -glucose absorption
by mannose, glycerol, glucosamine and unlabeled glucose.

Figure 9.

Inhibition of 2.5 mM ^{14}C -fructose absorption
by glucose, mannose, glycerol and glucosamine.

Figure 10.

Inhibition of ^{14}C -2-deoxyglucose absorption
by glucose and glycerol.

Figure 11.

Inhibition of 2.5 mM ^{14}C -mannose absorption
by glycerol, glucose and 2-deoxyglucose (D-2-D).

Figure 12.

**Inhibition of 2.5 mM glycerol absorption
by mannose and glycerol.**

Figure 13.

**Effect of fructose on the uptake of
2.5 mM glucose, mannose, glucosamine, glycerol,
or 2-deoxyglucose (D-2-D).**

Figure 14.

Effect of unlabeled substrates upon uptake of ^{14}C -carbohydrates by T. gambiense. Values are percent inhibition obtained at fixed inhibitor to substrate ratios of 16:1 (4:1 when fructose was the inhibitor). The range for all values is within ± 5 .

¹⁴ C-labelled Substrate				
Inhibitor	glucose	mannose	glycerol	fructose
glucose	84	83	56	91
mannose	86	82	63	92
glycerol	65	64	86	86
fructose	9	8	3	81

Figure 14-A.

Effect of unlabeled substrates on the absorption
of ^{14}C -glucose, fructose and glucosamine.

Values are percent inhibition obtained at inhibitor
to substrate ratios of 16:1 (4:1 when fructose was
the inhibitor).

The range for all values is within ± 5 .

¹⁴ C-labeled Substrate				
Inhibitor		Fructose	Glucose	Glucosamine
	Fructose	81	9	75
	Glucose	91	84	90
	Glucosamine	79	42	

Figure 15.

Effect of fructose on the
uptake of ^{14}C -glucose at 37 C.

S/I

A/I

Figure 16.

Inhibition of 2.5 mM ^{14}C -glucose or 2.5 mM ^{14}C -fructose
absorption by increasing concentrations
of glucosamine.

Figure 17.

Effect of glucose or fructose on the
absorption of ^{14}C -glucosamine by T. gambiense.

Figure 18.

Effect of glucose, fructose or N-acetyl-glucosamine
on the uptake of ^{14}C -glucosamine.

Figure 19.

Uptake of ^{14}C -N-acetyl-glucosamine
in the presence or absence of 5.0 mM glucosamine.

Figure 20.

Effect of glycerol, glucosamine, mannose and
unlabeled glucose on the uptake of
2.5 mM ^{14}C -glucose.

I = inhibitor concentration;

i = percent inhibition.

Figure 21.

Effect of some substrates on the
uptake of ^{14}C -glucose.

Figure 22.

Effect of some substrates on
the uptake of ^{14}C -mannose.

Figure 23.

Effect of some substrates on
the uptake of ^{14}C -mannose.

Figure 24.

Effect of mannose, glucose, or fructose on
the absorption of 2.5 mM ^{14}C -glycerol.

Figure 25.

Effect of N-acetyl-glucosamine (NAG), glucose
and fructose on the uptake of
 ^{14}C -glucosamine.

Figure 26.

Effect of some substrates on
the uptake of ^{14}C -fructose.

Figure 27.

Effect of some substrates on
the uptake of ^{14}C -fructose.