THE ABSORPTION OF SUGARS AND SODIUM

IN VITRO BY TILAPIA MOSSAMBICA

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PREFACE

I would like to acknowledge the help given to me by Spencer Malecha, graduate student employed by the Co-operative Fisheries Unit of the University of Hawaii, who assisted in the collection and identification of _Tilapia_ _mossambica_.

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ABSTRACT

The absorption of ^{14}C sugars and ^{22}Na has been studied
in the cichlid <u>Tilapia</u> <u>mossambica</u> and the surgeonfish <u>Acan-
thurus</u> <u>sandvicensis</u> using the everted sac technique of
Wilson and Wiseman (1954). Some of the parameters of glu-
cose transport were investigated and it was found that the
amount of glucose transport is directly related to the
length of time of incubation, the amount of gut tissue, and
the level of endogenous tissue glucose. The fresh or salt
water environment in which the fish were raised or the diet
which the fish were fed did not affect glucose transport.
Surgeonfish transported glucose faster than tilapia, in-
dicating that differences in glucose transport between fish
are species specific.

The possibility of a common carrier for sodium and glu-
cose transport was investigated in tilapia, and a direct re-
lationship between the sodium concentration of the medium
and glucose transport was found when the sodium level of the
medium was below 100 meq/L. Countertransport of endogenous
glucose was inversely related to the sodium concentration
below 100 meq/L and occurred before a reversal of the sodi-
um gradient was possible. The sodium and osmotic pressure
levels of the blood and intestinal fluids were about the
same, and the sodium level was slightly higher than the
level required for maximal transport of glucose <u>in</u> <u>vitro</u>.

Different areas of the gut transported glucose and sodium at different rates. The poisons KCN and ouabain were found to inhibit glucose transport, but they did not seem to affect sodium transport. An increase in the transport of glucose, but not sodium, occurred with increasing mucosal glucose concentrations. The possibility of a 1:1 stochiometry for glucose and sodium transport could not be eliminated, however, because of the high level of sodium transport regardless of the presence or absence of glucose. It was concluded that in fish glucose and sodium do not share the same transport mechanism as proposed by Crane (1965).

The results of the study of the relationship between glucose transport and enzyme systems in tilapia indicate that sugar transport is very specific and that enzymes are probably involved. D-Glucose and D-galactose were rapidly transported while D-zylose, D-fructose, D-mannose, L-fucose, D-glucuronic acid, D-gluconic acid, and D-mannitol did not enter the serosal sac faster than could be accounted for by diffusion. Galactose was found to be a non-competetive inhibitor of glucose transport only when in the mucosal fluid, indicating that the rate limiting part of the enzyme system is on the luminal or mucosal surface. The rate of glucose transport was affected by the pH of the medium, which also indicates that enzymes of the mucosal surface may be involved in glucose transport.

In conclusion, a model of glucose transport is presented where the first step in transport is the binding of glucose to enzymes on the mucosal surface and where at least a part of the energy necessary for glucose transport is derived from the metabolism of glucose itself.

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I. INTRODUCTION

REVIEW OF THE LITERATURE

The literature concerning the intestinal absorption of sugars is mostly concerned with mammals and has been comprehensively reviewed by Crane (1960), Faitelberg (1961), Wilson (1962), Schoffeniels (1964), Csaky (1965), and Benson and Rampone (1966).

Recent papers which are not covered by the reviews and which are important to this thesis are those by Alvarado and Crane (1964), Alvarado (1965, 1966A, and 1966B), Asano (1964), Baily et al (1965), Bassler et al (1966), Bingham et al (1966), Bosakova and Crane (1965A and 1965B), Brown and Moog (1967), Clausen (1965), Crane and Lyon (1966), Crane et al (1965), Curran (1965), Dahlqvist and Nordstrom (1966), Dawsen et al (1965), Duerdoth et al (1965), Huang and Rout (1967), Lyon and Crane (1966 and 1967), Pope et al (1966), Rosenweig et al (1965), Saunders and Isselbacher (1965, Schatzmann (1965), and Schultz and Zalusky (1965).

The general pattern of absorption of sugars in mammals that has emerged in the last few years as discussed in the review articles and in the afore mentioned papers is as follows.

Glucose and galactose are actively absorbed along with a few other synthesized sugars with a similar configuration. All actively ansorbed sugars are thought to share the same pathway or carrier.

Maltose is absorbed faster than can be accounted for by luminal intestinal digestion and absorption as monosaccharides. The distribution of disaccharidases coincides with the areas of monosaccharide absorption in the gut.

The active absorption of sugars requires and is sensitive to varying amounts of ionic sodium.

There is a change of potential during active transport of sugars across the intestinal membrane which is associated specifically with sodium ion.

The energy for the active transport of sugars is thought by Crane (1965) and others to come from sodium gradient created when the coupled sodium-carrier-glucose enters the cell. Csaky (1965) suggests that the energy comes directly from oxidative phosphorylation.

Because of the specificity of the active transport process an enzyme or enzymes are presumed to be involved.

Poisoning experiments with the glycoside phlorizin, which is a structural analog of glucose, indicate that the enzyme or carrier is on the luminal mucosal surface of the epithelial cells.

Keston (1963) and Bailey and Penchev (1964) have implicated specific enzymes, such as mutarotase, as being involved in active transport of sugars.

The following studies of active transport in lower vertebrates indicate that the absorption of sugars proceeds in much the same manner as in mammals.

The transport of sugars in turtles has been studied by Fox (1961A, 1961B, 1962, and 1965), Fox and Mussachia (1960), and Fox et al (1964).

Cordier and Worbe (1954, 1955, 1956A, and 1956B), Csaky and Fernald (1960), and Lawrence (1963) have studied the absorption of sugars in amphibians.

Absorption of sugars in fish has been studied by Carlisky and Huang (1962), Cordier (1953 and 1955), Cordier et al (1957), House and Green (1963), Hollands and Smith (1964), Huang and Rout (1967), Mussachia (1960 and 1961), Mussachia and Fisher (1960), Mussachia and Hines (1963), Mussachia and Westhoff (1962A, 1962B, and 1963), Mussachia et al (1961, 1964, 1966), Rout et al (1965), Smith (1964), Stokes and Fromm (1964 and 1965), and Wilson (1957).

One of the major problems in studying sugar transport in fish has been the large variability found in fish within experiments, between experiments, and between different fish. PARAMETERS OF TRANSPORT: The parameters of intestinal glucose transport in fish, especially those used to quantify data, have varied greatly from one worker to another. Almost every possible combination of glucose transport per unit wet weight, dry weight, and length has been used. For instance, wet weight has been used by Rout et al (1965), Huang and Rout (1967), and Carlisky and Huang (1962), dry weight by Mussachia et al (1965) and Stokes and Fromm (1964), and length by Smith (1964).

None of these workers appear to have investigated whether the methods used to quantify data decrease variability between determinations or are related in some way to the rate limiting step in glucose transport.

ENDOGENOUS GLUCOSE: Previous studies of glucose transport in fish have been made using reducing sugar or glucose oxidase determinations instead of glucose ^{14}C to estimate glucose transport. Therefore, it is impossible to separate endogenous glucose from the gut tissues from mucosal fluid glucose transport across the membrane. Mussachia et al (1965) evaluated endogenous glucose using blank preparations and concluded that up to 20% of their transport rates might be due to endogenous glucose.

EFFECT OF ENVIRONMENT: Active absorption of glucose and galactose or synthesized sugars resembling glucose, such as 3-methyl-glucose, has been demonstrated in all groups of lower vertebrates, but numerous exceptions have been found in fish, as noted below.

Active glucose transport across the intestinal membrane of fish was found by Carlisky and Huang (1962), Cordier and Chanel (1953), and Cordier and Maurice (1955). Mussachia and Fisher (1960) found that glucose was actively absorbed in two species of fresh water fish but not by four species of salt water fish. Wilson (1957) found that glucose was not actively absorbed in the puffer, a marine fish. Subsequently, Mussachia et al (1966) demonstrated active

transport of glucose for the scup and the puffer that had been previously shown not to transport glucose by Mussachia and Fisher (1960), and Wilson (1957). Rout et al (1965) were unable to show active absorption of glucose in the flounder.

The question of whether a salt or fresh water environment affects glucose transport in fish remains unanswered, since this experiment has not been carried out.

Similarly, the effect of a fresh or salt water environment on sodium transport has not been worked out.

House and Green (1963) studied the movement of sodium and water acrodd the intestine of a marine fish using ^{22}Na and found : positive net movement of sodium into the serosal fluid.

Smith (1964) studied sodium movement _in_ _vitro_ in the fresh water goldfish using flame spectrophotometry. He found that there was a loss of sodium from the everted intestinal sac that paralleled the water loss so that sodium concentration/ml remained the same.

DIET AND GLUCOSE TRANSPORT: The effect of a galactose diet upon glucose transport was studied by Westenbrink (1936) who found that galactose in the drinking water of rats increased glucose transport many fold. No other workers have followed this valuable lead to investigate this apparent example of enzyme induction in glucose transport.

SODIUM REQUIREMENT FOR TRANSPORT: No studies have been made of the ionic requirements for glucose transport in fish gut, but Smith (1964) indicates that he found an increase in potential difference across the gut when he added glucose to the preparations. This is in keeping with the findings of Schultz and Zalusky (1964) in rabbits, and it indicates that sodium is involved in glucose transport in fish.

GLUCOSE AND SODIUM TRANSPORT: The relationship between sodium and glucose transport has not been studied in fish.

Using hamsters, rabbits, and rats, Crane (1962), Schultz and Zalusky (1964), Kipnis and Parrish (1965), Curran (1965), Crane et al (1965), and Lyon and Crane (1966 and 1967) all present strong evidence, based on potential difference, for the direct involvement of sodium ion with glucose. Crane et al (1965) indicate that the energy for this glucose transport system apparently comes from the entry of sodium and glucose into the cell attached to the same carrier molecule. The cell interior, being negative, provides the motive force. The glucose and sodium dis-associate and sodium is pumped back out to maintain the intra-cellular gradient leaving the glucose on the interior. The carrier molecule can now migrate back to its original position.

Csaky (1965) concludes, however, that sodium is linked to glucose transport via the energy system directly.

Barry et al (1965) present evidence that the electrical

phenomena are related to hexose transfer and not to sodium transfer. The work of Barry et al (1965) has forced Lyon and Crane (1967) to modify their theory somewhat.

AREAS OF TRANSPORT: Absorption of glucose normally occurs only in the small intestine and not from the stomach and large intestine of most invertebrates according to Crane (1960). Mussachia and Neff (1963) found uniform rates of absorption of glucose in the areas studied in the gut of the ground squirrel. Glucose absorption has not been studied throughout the gut of fish.

House and Green (1963) and Smith (1964) have studied sodium transport in fish in the upper intestine, but the rate of absorption of sodium throughout the gut of fish has not been studied.

No one has made a simultaneous study of glucose and sodium absorption throughout the gut of any animal.

There have been no studies on the effect of poisons such as ouabain and KCN throughout the length of the gut to separate areas of active transport from areas of diffusion of glucose and sodium.

ENZYMES AND TRANSPORT: Some of the possible parameters of the carrier enzyme in fish have already been studied by Cordier and Maurice (1955), Carlisky and Huang (1962), Mussachia et al (1966), and Huang and Rout (1967). Their results indicate that temperature increases the rate of glucose and galactose transport.

The influence of mucosal glucose concentration on glucose transport has been studied in fish by Carlisky and Huang (1962), Smith (1964), and Stokes and Fromm (1964). They found that increased glucose concentration caused increases in glucose absorption that could be fitted into Michaelis Menten equations. Huang and Rout (1967) found that increased mucosal galactose concentration increased galactose transport at 26° C but not at 16° C.

Inhibition of glucose transport using phlorizin, ouabain or other inhibitors has been studied in fish by Carlisky and Huang (1962), Smith (1964), and Mussachia et al (1965). All inhibitors were found to inhibit active glucose transport. Huang and Rout (1967) found that galactose transport was similarly inhibited by phlorizin.

Hollands and Smith (1964) studied distribution of phosphatases of goldfish intestine. They were unable to relate these enzymes specifically to active glucose transport.

The specificity of sugar transport in fish was studied using 11 different non-radioactive sugars in the killifish by Huang and Rout (1967) with equal concentrations of sugar in the serosal and mucosal fluids at the start of the experiment. They found that D-glucose, D-galactose, 3-0-methyl-D-glucose, L-mannose, and L-Zylose were transported against a concentration gradient while L-glucose, D-mannose, D-zylose, a-methyl-glucoside, D-sorbose, and D-fructose were

not transported against a gradient.

Because they did not use ^{14}C sugars in their work it is not possible to separate endogenous glucose from the test sugars.

No one has studied the effect of serosal glucose on glucose transport to see if metabolism of glucose contributes to glucose transport.

The effect of serosal and mucosal galactose on glucose transport in fish is unknown.

No one has studied the effect of pH on glucose transport to determine whether there is a pH optimum for glucose transport.

MODELS OF TRANSPORT: Models of glucose transport that involve glucose, sodium, and a mobile carrier molecule have been presented for intestinal glucose transport in warm-blooded vertebrates by Crane (1960, 1962, and 1965), Schultz and Zalusky (1964), Barry et al (1965), and Benson and Rampone (1966).

Rout and Huang (1967) hypothesize that D-glucose is exchanged across the gut for L-glucose in fish. L-glucose is not known to occur anywhere as a natural sugar according to Bell (1962).

There is no model of glucose transport in the literature which can account for the specificity of sugar transport, which presumably requires a large protein enzyme, the requirement of the transport system for sodium, the energy

source for transport, and the distribution of active transport activity throughout the gut.

STATEMENT OF THE PROBLEM:

It should be clear from the preceding discussion of the literature that there is a great deal that is not known about glucose and sodium transport in fish. The study of glucose and sodium transport, using glucose ^{14}C and ^{22}Na, which forms the basis of this dissertation attempts to fill the gaps in our knowledge of glucose and sodium transport in seven major areas: the parameters of transport, the effect of environment on transport, the effect of diet on transport, the level of sodium required for maximal glucose transport, the inter-relationship between glucose and sodium transport, the areas of transport throughout the gut, and some of the enzymatic parameters of glucose transport.

In addition, a model of glucose transport is presented in an attempt to account for and compare the results of this study with tilapia and the results that others have found, working both with fish and other vertebrates, in a form that allows an overall visual comparison.

II. METHODS AND MATERIALS

Tilapia mossambica was introduced into several of the brackish and fresh water areas of Oahu by the Hawaii State Fish and Game Department, both deliberately and accidently, from 1952 to 1966. The tilapia have thrived and form the dominant fish species of fresh, brackish, and salt water ponds, canals, and lakes.

Tilapia were collected from three areas: the sea water canals that provide drainage for Hickam Air Force Base near Honolulu International Airport, Enchanted Lake (a small, brackish water lake in Kailua), and from the tuna bait hatchery at Sand Island by means of throw nets or dip nets.

The tilapia were transported to the University of Hawaii and placed in 50% sea water. To prevent mass mortalities the fish were treated for three days with commercial preparations (Aqua Biotics, distributed by Aquarium Pharmacuticals) containing 10 mg streptomycin sulphate, 10 mg merbromin, 10 mg Neomycin sulphate, and 27 mg copper sulphate per 5 gallons of aquarium water. Two other preparations were also used containing $C_{23}H_{14}CIN_2$ (Nox-Ich, distributed by Weco Products) and Na_2SC_3, $C_{12}H_{14}N_4O_2S$, and $C_{16}H_{18}CIN_3S_3H_2O$ (Sulacin, distributed by Weco Products). Mortalities averaged between 20 and 30 per cent for treated fish versus 80 to 90 per cent for untreated fish. It was observed that most of the treated fish that died had

been gilled in capture, causing scale loss across the top of the head.

After three days treatment in the 50% sea water and antibotics solution, the remaining fish were transferred to either fresh or salt water.

The fish were kept in a 50 gallon fiberglass aquaria and fed 0.5 oz (volume) of Clarkes Complete Trout Fingerling Pellets apiece each day. The pellets contained 37% crude protein, 5.5% crude fat, 7% fiber, and all essential vitamins and minerals. Tilapia were kept a minimum of one week before they were used for experimentation.

Two groups of five fish kept in sea water were fed 1.0 grams a day of galactose apiece (dissolved in a gel of agar and trout pellets) for two weeks to see if the galactose would increase the rate of glucose transport, as has been reported for rats by Westenbrink (1936).

The surgeonfish _Acanthurus_ _sandvicensis_ was collected from the outer edge of the coral reef directly in front of the Waikiki Marine Laboratory by skin diving at night using underwater lights and dip nets. They were kept alive in nylon mesh bags until placed in porous live-bait buckets for the trip to the University sea water room in Edmondson Hall.

The surgeonfish were placed in circulating 50 gallon fiberglass aquaria for 36 hours prior to experimentation to allow them to defecate fecal material so that the intestines could be more easily everted. No mortalities occurred in

these fish.

Fish to be used for experiments were killed with a sharp blow between the eyes with a lead pipe and opened up from the anus to the gills. A second incision from the anus to the backbone and anterior to the gills, cutting through the ribs, exposed the whole of the gut when the flap of flesh was lifted upwards and towards the head.

The upper intestine of tilapia is in intimate association with a lobe of the liver, as can be seen in Figure 1. Except for these experiments which studied the absorption of glucose throughout the gut, this was the area that was used for all experiments. This area was chosen because preliminary studies had indicated that it was an area of active absorption, it was easily accessible, it could be removed without excessive damage or stretching, and because it was possible to select the same area of the gut from fish to fish, regardless of the size of the fish. The length of this segment of the gut varied from three to nine centimeters, depending upon the size of the fish used in the experiments.

Fish were selected for individual experiments according to size. Every attempt was made to eliminate variation in experiments by eliminating variations in the size or condition of the fish.

The intestinal segments were cut $\frac{1}{2}$ cm below the point of junction with the stomach and at the end of the attachment of the intestine with the lobe of the liver. After
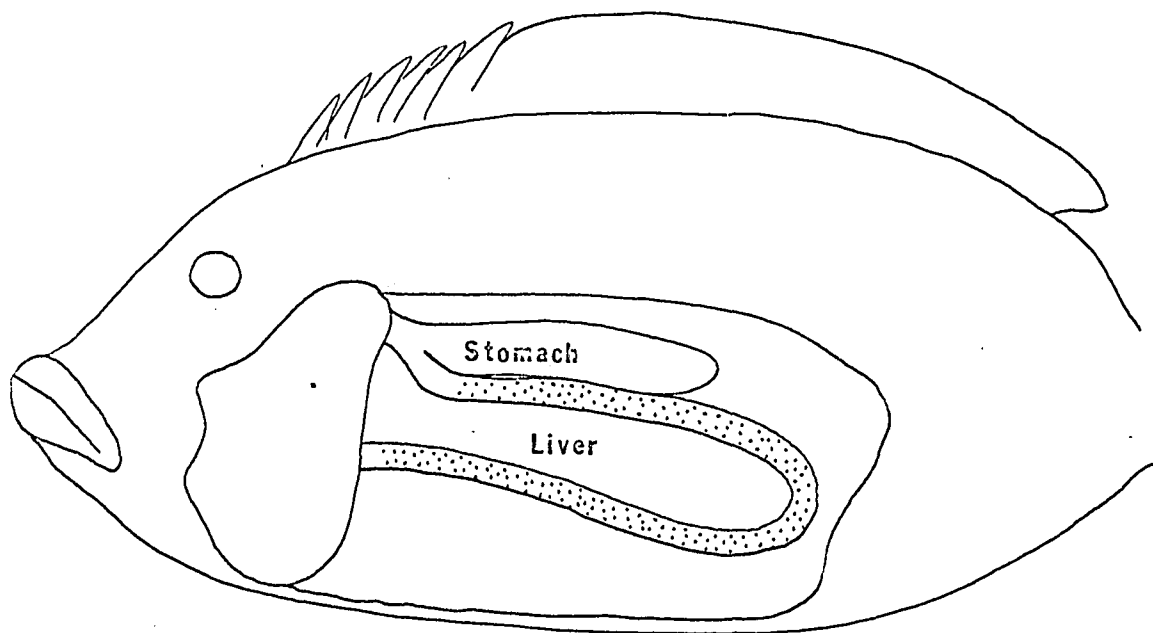
FIGURE 1.   A DIAGRAMATIC VIEW OF THE GUT

The stipled area indicates the segment
of the gut intimately related to a lobe
of the liver and used in all experiments
to form the everted intestinal sac.

removal from the fish, the intestinal segment was rinsed in tilapia Ringers solution containing 6.02 g NaCl, 0.4 g NaHCO$_3$, 0.7214 g CaCl$_2$, and 0.3728 g KCl per liter (pH 7.74), worked out previously for tilapia by Hiat et al (1956).

The segments were everted on a piece of capillary glass tubing according to the method of Mussachia et al (1966). After eversion the segments were rinsed again, tied off at one end, and filled with 0.5 ml of tilapia Ringers by means of a blunted needle and syringe. The filled segments were checked for leaks and then placed in the incubation medium. A diagramatic view of an everted sac is presented in Figure 2.

The intestine of A. sandvicensis is not in intimate association with the liver as in tilapia, but the corresponding area of the intestine was used in these experiments and the procedure was identical with that of tilapia.

No pre-incubation period was used to reduce variability in any of the experiments as most other workers have done because one of the purposes of this thesis was to investigate the causes of variability. Besides, it was felt that if glucose transport was dependent upon oxidative phosphorylation, as indicated by the effect of cyanide upon the system, pre-incubation might well have increased variation due to waste metabolic build up or cell death.

The everted gut segments were incubated in 25 ml test
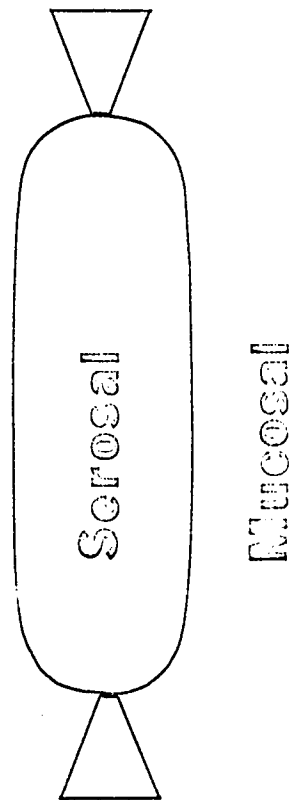
FIGURE 2.   A DIAGRAM OF THE EVERTED GUT SAC

tubes filled with 5 ml of tilapia Ringers (the mucosal
fluid) and aereated by means of a xontinuous stream of
95% O_2/5% CO_2 bubbles released from plastic tubing at the
bottom of the test tube. The constant agitation produced
by the bubbles and the high oxygen content of the gas in-
sured that the tissues would receive adequate oxygen.

After incubation of the everted gut segments for a pre-
determined time period (usually one hour), the everted sacs
were removed from the test tubes, the length measured to
the nearest tenth of a centimeter, blotted dry, and the
serosal fluid removed using a one ml tuberculin syringe and
a number 20 needle.

The now-empty sacs were again blotted dry and the wet
weight of the sac was determined to the nearest hundredth of
a gram. In cases where the dry weight was also determined,
the tissue was placed on pre-weighed planchetts, dried for
6 hours at 100° C in an electric oven, and cooled for one
hour in a vacuum desicator. The dry weight was determined
to the nearest ten thousandth of a gram on a torsion balance.

A control experiment using the same procedure without
everting the gut segments was run to determine whether glu-
cose ^{14}C moved from the serosal to the mucosal surface. No
glucose ^{14}C was detectable in the mucosal fluids in this
experiment.

The mucosal incubation medium was varied according to

the experiment, but except as noted below, it consisted of 5 ml of tilapia Ringers, 0.2 mg/ml (20 mg per cent) D-glucose, and 10 lambda of glucose U-^{14}C solution containing approximately 0.0002 mg glucose (3.3 uc). The temperature during the experiments varied between 22 and 24°C.

The effect of glucose concentration upon absorption of glucose and the inhibition of glucose transport by galactose was studied using 5 ml of tilapia Ringers containing 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, or 2.0 mg/ml solutions of glucose in the mucosal fluid plus 10 lambda of glucose U-^{14}C. In the galactose inhibition experiments the levels of mucosal galactose were 1 g/L and 10 g/L.

The influence of pH on glucose transport was studied using a modification of the constant ionic strength buffer taken from Long (1965) using Na_2HPO_4 and NaH_2PO_4. It was important to keep the amount of sodium constant in these experiments because it was found that glucose transport was dependent upon sodium ion level. The pH of the solutions varied from 5.6 to 8.0, as measured with a Beckman pH meter. The serosal and mucosal fluids were the same pH at the start of the experiment, and the pH of the mucosal fluid was found not to vary significantly during the one hour period of incubation.

The relationship of the sodium ion to the transport of glucose from the mucosal fluid into the serosal sac was studied using 2, 4, 6, or 8 grams of sodium chloride added

to one liter of a base solution containing 0.4 g $NaHCO_3$, 0.7214 g $CaCl_2$, and 0.3728 g KCl per liter. The pH of these solutions was constant at pH 7.70. The serosal and mucosal fluids were the same at the start of the experiment so that an initial sodium gradient would not complicate analysis of the results.

In half the experiments using sodium ion concentration below that of the blood, the incubation solutions were made isotonic with the blood by adding sucrose. This was done to check on the effect of lowered osmotic pressure on glucose transport. These same solutions were used to study the efflux of tissue glucose due to lowered sodium levels.

In poisoning experiments the standard incubation medium was used with either 4 x 10-4 M ouabain or 1 x 10-3 M NaCN (final concentrations). These concentrations were picked because they are known to be inhibitory to glucose transport in other animals according to Crane (1960) and Smith (1964).

In experiments where other ^{14}C sugars were used the mucosal medium was always 5.55 meq (1 mg glucose) in 5.0 ml of tilapia Ringers plus 10 lambda of the sugar isotope. The serosal fluid volume at the start of the experiment was always 0.5 ml of tilapia Ringers.

The serosal medium was always of the same ionic concentration and pH as the mucosal fluid. The initial volume of the serosal fluid was kept constant at 0.5 ml. This

volume was large enough to provide the necessary aliquots for analysis and small enough so that there was no distension or stretching of the everted segments. No sugars or isotopes were placed in the serosal medium except as noted below.

The effect of glucose or galactose in the serosal fluid upon glucose transport from the mucosal fluid into the serosal sac was studied by placing 0.5 ml of tilapia Ringers containing 0.2, 0.4, 0.8, 1.2, or 1.6 g/L glucose or galactose inside the serosal sac. The everted gut segment was then incubated for one hour in 5 ml tilapia Ringers containing 0.2 g/L glucose plus 10 lambda glucose U-^{14}C.

Glucose transport into the everted serosal sac was also studied using the method of Jorgenson et al (1961) that allowed duplicate runs using the same everted segment to reduce variations between different gut preparations, but it was found that variations due to loss of transport ability with time out-weighed the advantage of paired comparisons. It was also technically more difficult to set up the everted gut segment on the apparatus and a large number of the segments were torn in the process.

Glucose transport into the everted intestinal sac was determined using D-glucose U-^{14}C purchased from New England Nuclear Corporation and CalBiochem. Samples (0.1 ml) were taken of the mucosal fluid at the start and end of each experiment and at the end of the experiment from the serosal

fluid (by means of a needle and syringe), placed upon pyrex glass planchetts, evaporated to dryness on a steam bath, and counted on a decade scaler produced by Radiation Counter Laboratories (model 10A). Total reducing sugars were determined by the method of Berger and Reynolds (1958) colorimetrically, which measures the reduction of the yellow ferricyanide ion.

Tissue glucose ^{14}C was determined by double extraction of the intestinal segments with 4 ml of 80% ethyl alcohol. The alcohol was decanted into 25 ml beakers which were evaporated to dryness over steam. The dried residue was dissolved in 0.5 ml of cold distilled water, placed on watch covers and evaporated to dryness over steam. The residue was dissolved in 0.1 ml of distilled water and 10 lambda was spotted on Whatman no. 1 paper for chromatography. Ten lambda of carrier glucose (containing 10 ug of glucose) was over-spotted and dried. The papers were run in N-butanol/ pytidine/water (3/2/1.5) in ascending chromatography, dried, and developed with P-ansidine-CHl (saturated solution in Butanol) according to the method of Kabat and Meyer (1966). The papers were counted on both the decade scaler and a nuclear Chicago strip counter and standards were run to correct for attrition of glucose ^{14}C and non-glucose ^{14}C background. The results were calculated in terms of micromoles of glucose per 0.1 g of tissue (wet weight).

Glycogen was extracted using the cold 10% tri-chloracetic acid of Roe et al (1958). After precipitation of

glycogen with ethyl alcohol the glycogen was re-dissolved in 1.0 ml hot water, placed on pyrex planchetts, evaporated to dryness over a steam bath, and counted on the decade scaler. The results were calculated in terms of total micro-moles of glucose ^{14}C glycogen per 0.1 tissue (wet weight).

Other ^{14}C labeled sugars were studied in the same way as glucose ^{14}C. All of the sugars were checked for purity and to determine the products of absorption by means of paper chromatography using the same system used for tissue glucose.

Sodium transport was studied using ^{22}Na (purchased from Nuclear Chicago Corporation) in the same way as glucose ^{14}C. Sodium concentration in serosal, mucosal, intestinal, and blood fluids was determined using the Beckman DU flame spectrophotometer (using internal standards) according to directions in Beckman Manual 259B.

Osmotic concentration of the blood, intestinal, serosal and mucosal fluids was done by means of a Fiske Osmometer (model 9-62) with a small volume attachment.

In experiments where both ^{22}Na and glucose ^{14}C were used at the same time the activity of both was estimated using a 10 mg density aluminum shield. Two counts were taken for the unknowns: shielded and unshielded. A control experiment was run using known amounts of ^{22}Na and a standard graph of ^{22}Na activity loss due to shielding was

prepared. Glucose ^{14}C was found not to contribute signif-
icantly to the activity of the shielded counts. The total
^{22}Na activity was calculated using the graph of ^{22}Na act-
ivity loss due to shielding and the shielded count for the
unknown. The ^{14}C activity was calculated by subtracting
the total ^{22}Na activity taken from the graph from the
total unshielded counts for the unknown. Sodium and glu-
cose activity were calculated as micro-moles of sodium or
glucose per 0.1 g wet weight tissue, or in some cases as
micro-moles sodium or glucose per 1.0 cm of everted gut
sac length.

III. PARAMETERS OF TRANSPORT

RESULTS AND DISCUSSION

A great variability was observed in the data from the first experiment in this study of glucose transport in the everted intestinal segments of T. mossambica. Before subsequent studies on the effect of various treatments such as changing pH or substrate concentration could be carried out it was necessary to make a preliminary study of the parameters of glucose transport. The variability observed in preliminary experiments was found to be due to many factors. SEROSAL VOLUME: In the first experiments the everted intestinal sacs were filled with varying amounts of tilapia Ringers until the sac began to be distended according to the technique of Wilson and Wiseman (1954). This meant that larger segments contained more fluid than smaller ones. The effect of different volumes of serosal fluid in everted gut segments of approximately the same length can be seen in Figure 3.

Carlisky and Huang (1962), Rout et al (1965), and Wilson (1957) have used a constant volume to fill the everted intestinal sacs. Smith (1964) has filled them and Stokes and Fromm (1964) have used 10 ml of re-circulating fluid to fill the everted intestinal sacs. Since it is clear that the ability to concentrate glucose against a gradient remains relatively constant regardless of serosal volume and that the total amount of glucose transported
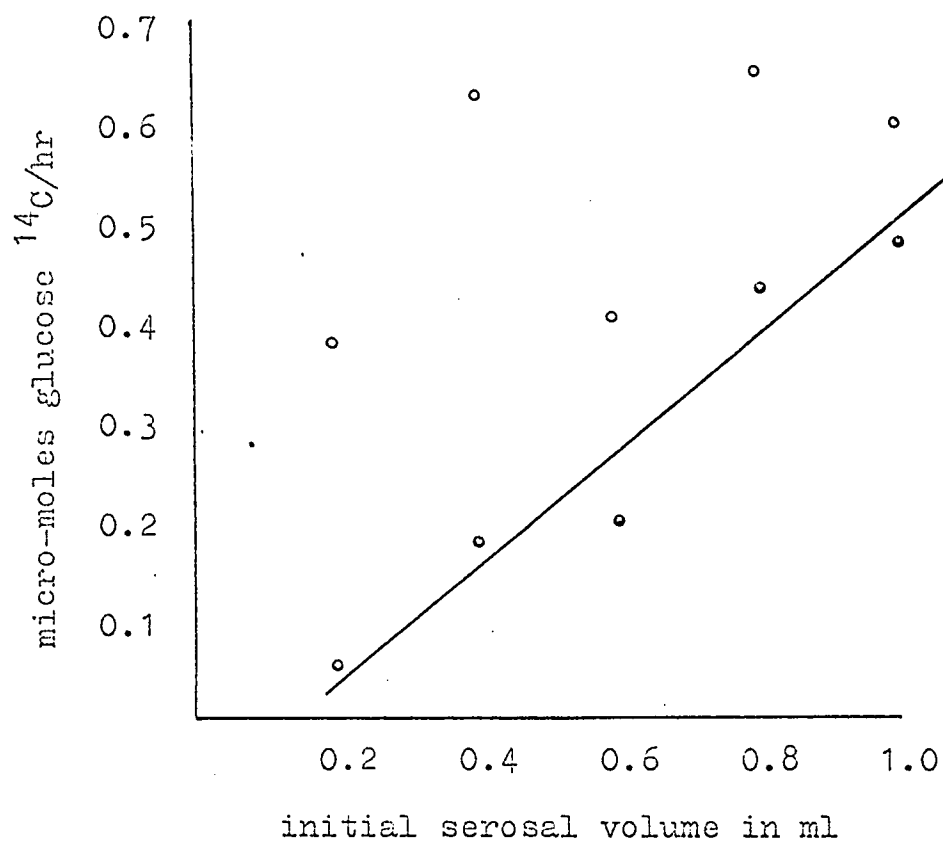
FIGURE 3. SEROSAL VOLUME AND GLUCOSE TRANSPORT

The effect of initial serosal fluid volume on glucose ^{14}C transport into the serosal sac for T. mossambica (0.2 g/L glucose in the mucosal fluid) for total glucose in micro-moles (•) and glucose concentration in micro-moles/ml (○).

into the everted gut sac depends upon serosal volume, the
method used should be considered when any comparison of
results is made.

ENDOGENOUS GLUCOSE: There can be no doubt that endogenous
tissue glucose contributes to the total serosal fluid glu-
cose when reducing sugar methods are used that eliminate
the effect of proteins liberated during the course of the
experiment.

In Figure 4 the total micro-moles glucose transported
into the serosal sac are plotted versus time for three
groups of twelve fish each. Group B everted segments trans-
ported almost one third as much glucose as did Group A,
even though there was no mucosal glucose available to Group
B. Group C, which had been starved one week prior to the
experiment, transported one half as much glucose into the
serosal sac as Group B and one fifth as much as Group A,
which had 0.2 g/L glucose in the mucosal fluid. It is clear
that endogenous glucose makes a significant contribution to
glucose transport into the serosal sac and that this con-
tribution varies significantly with the condition of the
fish. This may be the explanation for the variability that
Mussachia et al (1966) found in populations of fish from
year to year, although Crane and Mandelstam (1960) found no
difference in tissue uptake of glucose between starved and
fed hamsters.

In Table I the total micro-moles of glucose transported
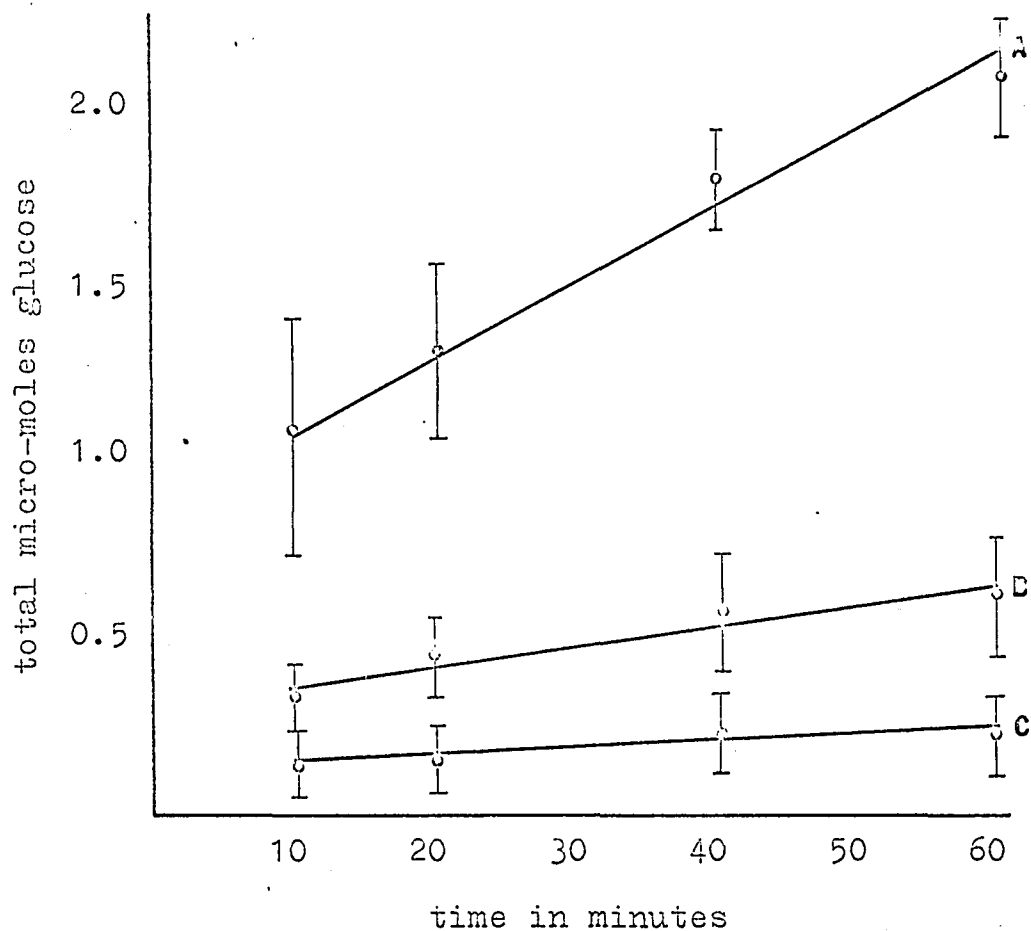
FIGURE 4.   EFFECT OF STARVATION ON ENDOGENOUS GLUCOSE

Total micro-moles glucose (in reducing equivalents) transported into the serosal fluid (1.0 ml) versus time for T. mossambica. A fed fish with 1 g/L glucose in mucosal fluid, B fed fish with no mucosal glucose, C starved fish with no mucosal glucose. Each point is an average for 3 fish.

TABLE I

TOTAL GLUCOSE VERSUS GLUCOSE ^{14}C TRANSPORT

Glucose ^{14}C transported into the serosal fluid com-
pared to the total micro-moles glucose in the serosal fluid
as determined in reducing equivalents of glucose (0.2 g/L
glucose in the mucosal fluid) for T. mossambica.

fish	micro-moles reducing value	micro-moles glucose ^{14}C	per cent
1	0.81	0.561	69.7
2	0.61	0.53	87.3
3	0.86	0.75	87.0
4	0.73	0.57	78.0
5	0.69	0.54	77.6
6	0.78	0.60	<u>74.4</u>
			80.0

into the serosal sac are compared for both reducing sugar and glucose ^{14}C in the same segment of gut. Glucose ^{14}C forms an average of 80% of the reducing sugar glucose. This indicates that endogenous glucose moves into the serosal sac regardless of whether there is glucose in the mucosal fluid or not. This is in close agreement with the data of Mussachia et al (1966) who found up to 20% of the glucose transport into the everted intestinal sac in the fish the scup was from endogenous glucose.

Reducing sugar or glucose oxidase determinations to measure glucose transport into the serosal sac have the obvious disadvantage that they cannot separate endogenous from mucosal glucose.

The effect of different concentrations of glucose in the serosal fluid on glucose transport from the mucosal to the serosal fluid can be seen in Figure 5. When the amount of glucose is increased within the sac one might expect that glucose would be transported into the serosal fluid more slowly because the glucose must be transported against a larger gradient. The results indicate that the more glucose that is initially present in the serosal fluid (i.e. the greater the gradient against transport) the more glucose is actually transported. The probable explanation for these results is that there is an equilibrium between the glucose initially present in the serosal fluid and tissue glucose, and that the more glucose that is initially placed in the
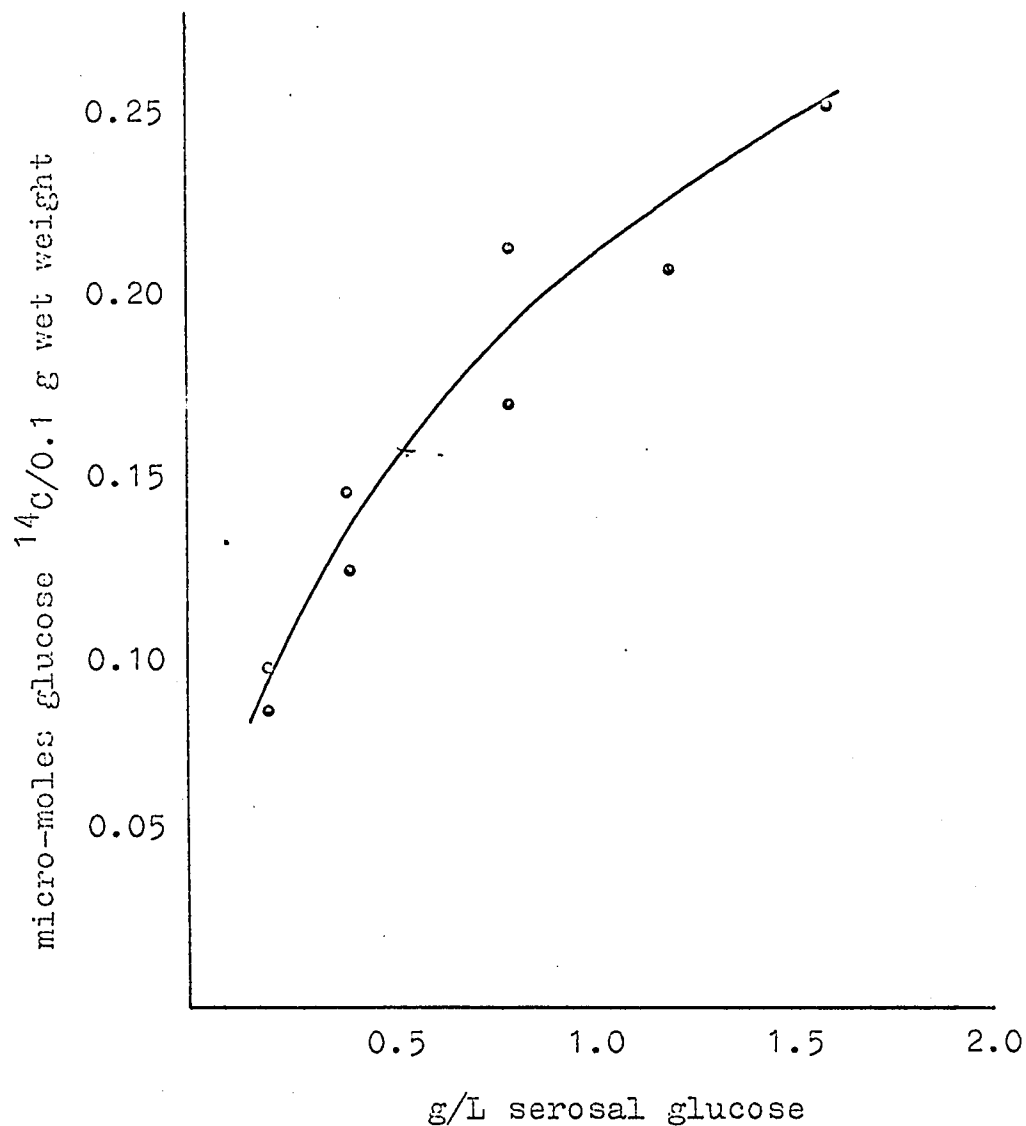
FIGURE 5.   SEROSAL GLUCOSE AND GLUCOSE ^{14}C TRANSPORT

Transport of glucose ^{14}C into the serosal sac per hour versus glucose concentration within the serosal sac at the start of the experiment (0.2 g/L in mucosal fluid) for T. mossambica.

serosal fluid, the more is available for metabolism by the
gut tissue.

In Figure 4 it can be seen that there is a difference
in the level of endogenous glucose between fed and starved
fish. If different levels of endogenous glucose have the
same effect on glucose transport as the glucose added to
the serosal sac by the investigator as seen in Figure 5, and
there is no good reason at this time to believe that it does
not, this endogenous glucose would have the effect of in-
creasing glucose transport into the serosal fluid.

The range of variation in glucose transport into the
serosal fluid in Figure 5 caused by glucose placed in the
serosal fluid by the investigator is 0.09 to 0.25 micro-
moles of glucose/0.1 g wet weight of tissue/hour. This
compares favorably with the normal range of glucose trans-
port, seen in Figure 12, of 0.11 to 0.28 micro-moles/0.1 g
wet weight of tissue/hour.

One of the causes of variation in glucose transport in
tilapia therefore is probably due to different levels of
endogenous glucose. This is in agreement with the results
of Stokes and Fromm (1964), who demonstrated higher rates of
glucose absorption and utilization for feeding trout.

QUANTIFICATION OF DATA: Data expressed in terms of total
micro-moles of glucose transported into the serosal sac, as
seen in Figures 3 and 4, is of limited value in comparisons
made between areas of the gut or in comparing the results in

experiments where different sized segments were used.

The usefulness of quantifying data in terms of wet weight of gut segments is investigated in Figure 6 and 7.

In Figure 6 the total micro-moles of glucose determined by reducing value transported into the serosal sac are plotted against the wet weight of the gut segments. A predominance of the points (five of nine) fall in almost a straight, horizontal line, but an analysis of linear regression using the method of Snedecor (1956) gives a probability of $P=0.05$. The std. dev. is 0.23 micro-moles/0.1 g wet weight.

The total micro-moles of glucose ^{14}C transported into the everted gut segment are plotted against wet weight of the gut segments in Figure 7. The linear regression of glucose on weight is statistically significant ($P=0.001$). The std. dev. was 0.0106 mg/0.1 g wet weight, significantly lower than the std. dev. for the regression of reducing glucose and wet weight.

The relationship beyween total micro-moles of glucose (as measured by reducing sugar values) and dry weight of the gut segments is shown on Figure 8 using gut segments from A. sandvicensis instead of tilapia. There is no statistically significant linear regression for this data.

In Figure 9 a paired comparison between the use of wet weight and dry weight to quantify the same data from four tilapia is presented. In the use of dry weight to quantify
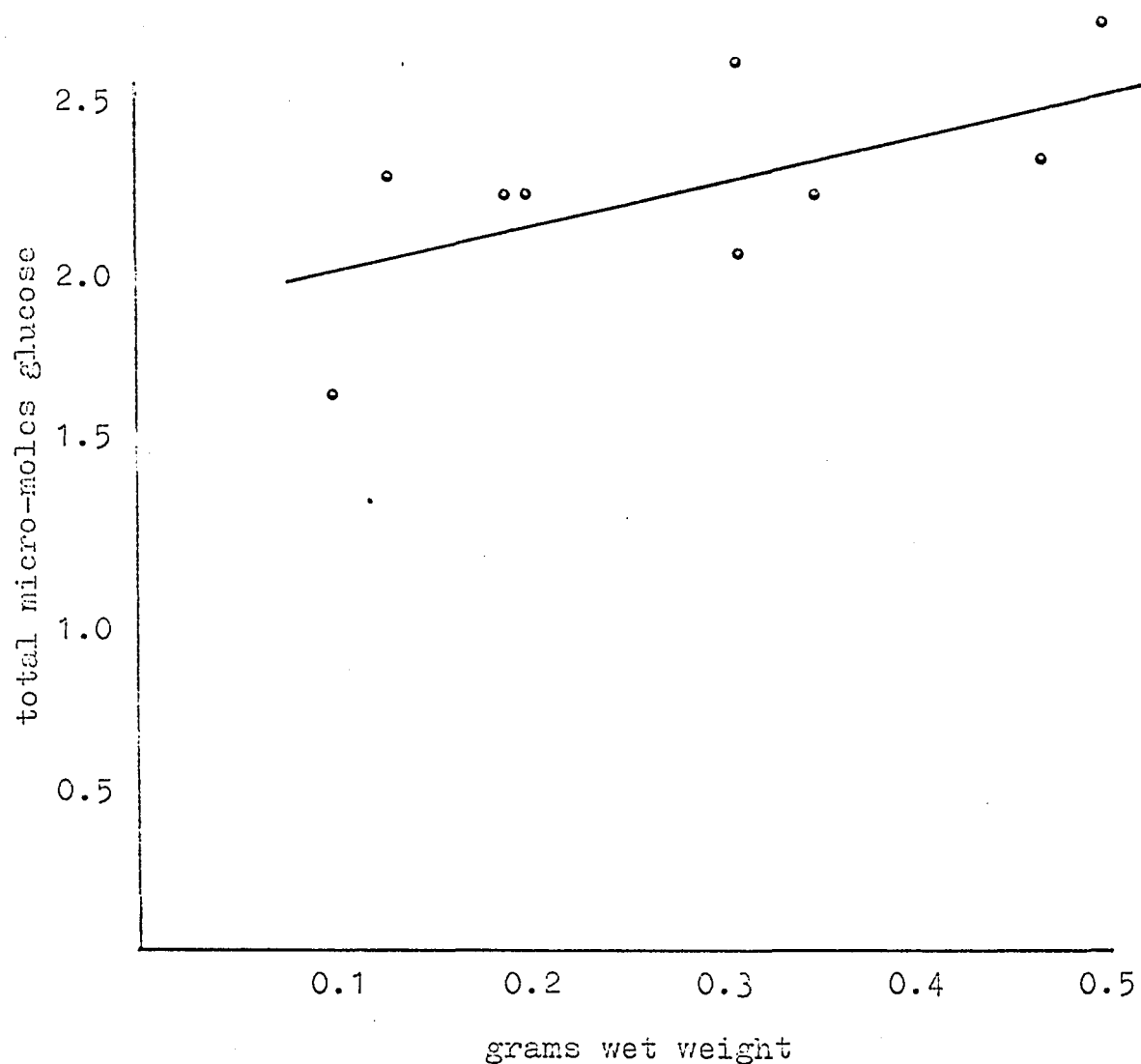
FIGURE 6.   GLUCOSE TRANSPORT VERSUS WET WEIGHT

Total micro-moles glucose (in reducing equiva-
lents) into the serosal fluid of T. mossambica
per hour versus wet weight of the gut segments
(1 g/L glucose in mucosal fluid; serosal vol-
ume 1.0 ml).
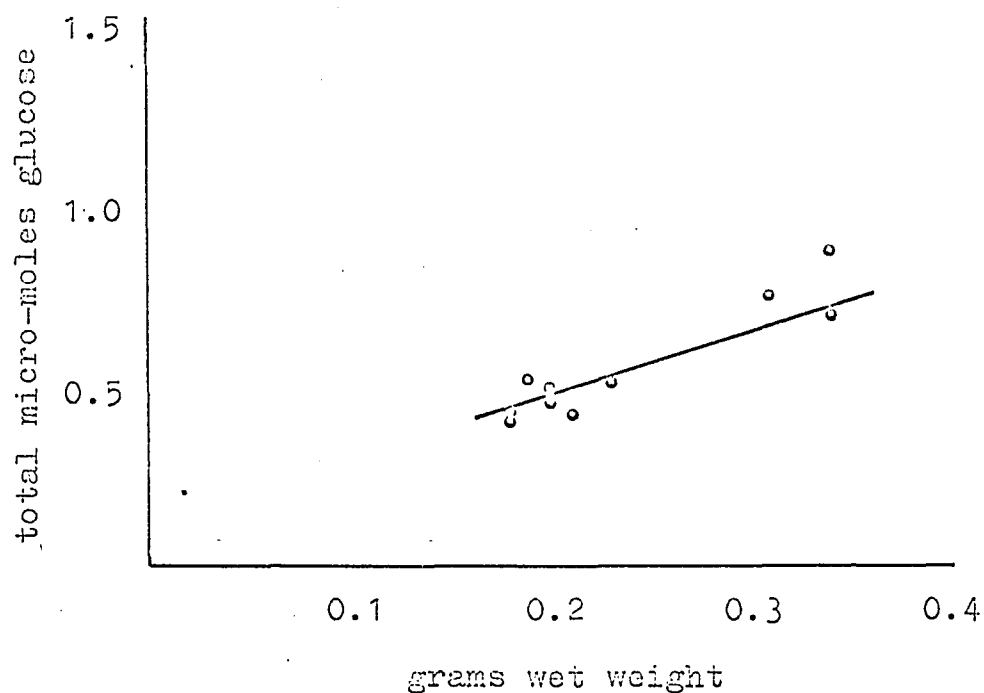
FIGURE 7.   GLUCOSE ^{14}C TRANSPORT VERSUS WET WEIGHT

Total micro-moles glucose ^{14}C transported into the serosal sac versus wet weight of gut tissue for T. mossambica (0.2 g/L glucose in the mucosal fluid).
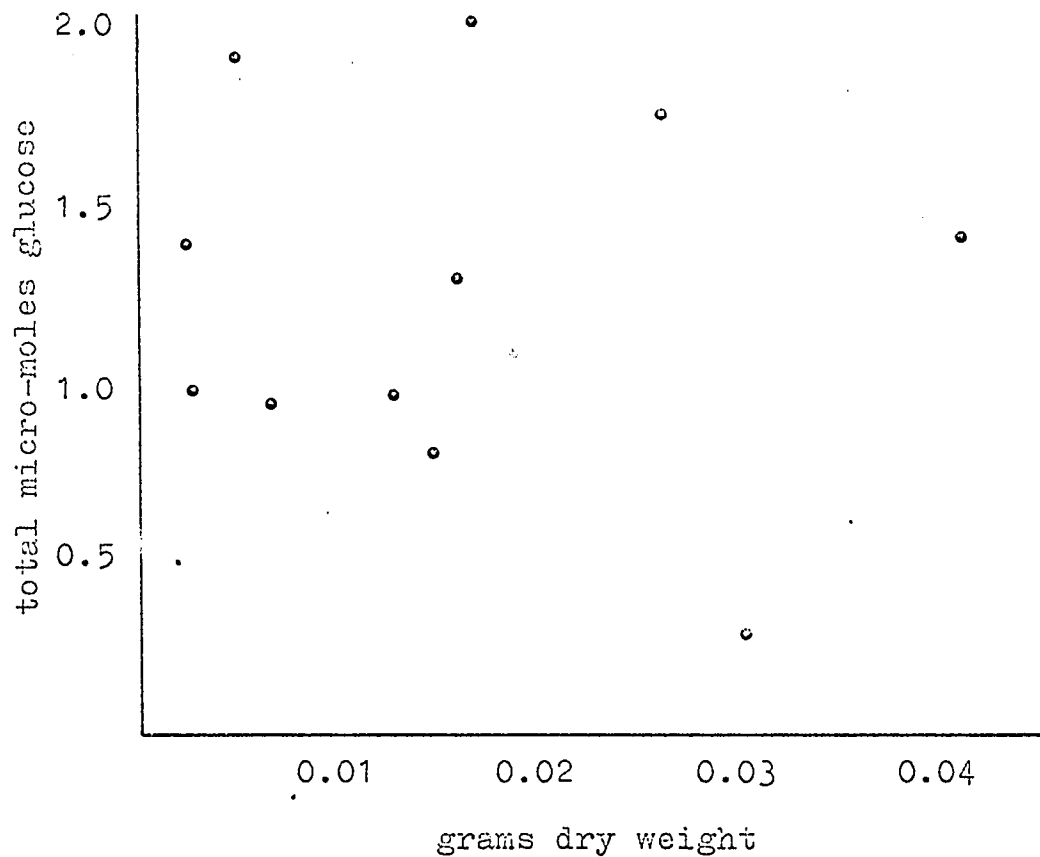
FIGURE 8.   GLUCOSE TRANSPORT VERSUS DRY WEIGHT

Total micro-moles glucose (in reducing equiva-
lents) transported into the serosal fluid of
A. sandvicensis versus dry weight of the ever-
ted gut segment (1 g/L glucose in the mucosal
fluid).
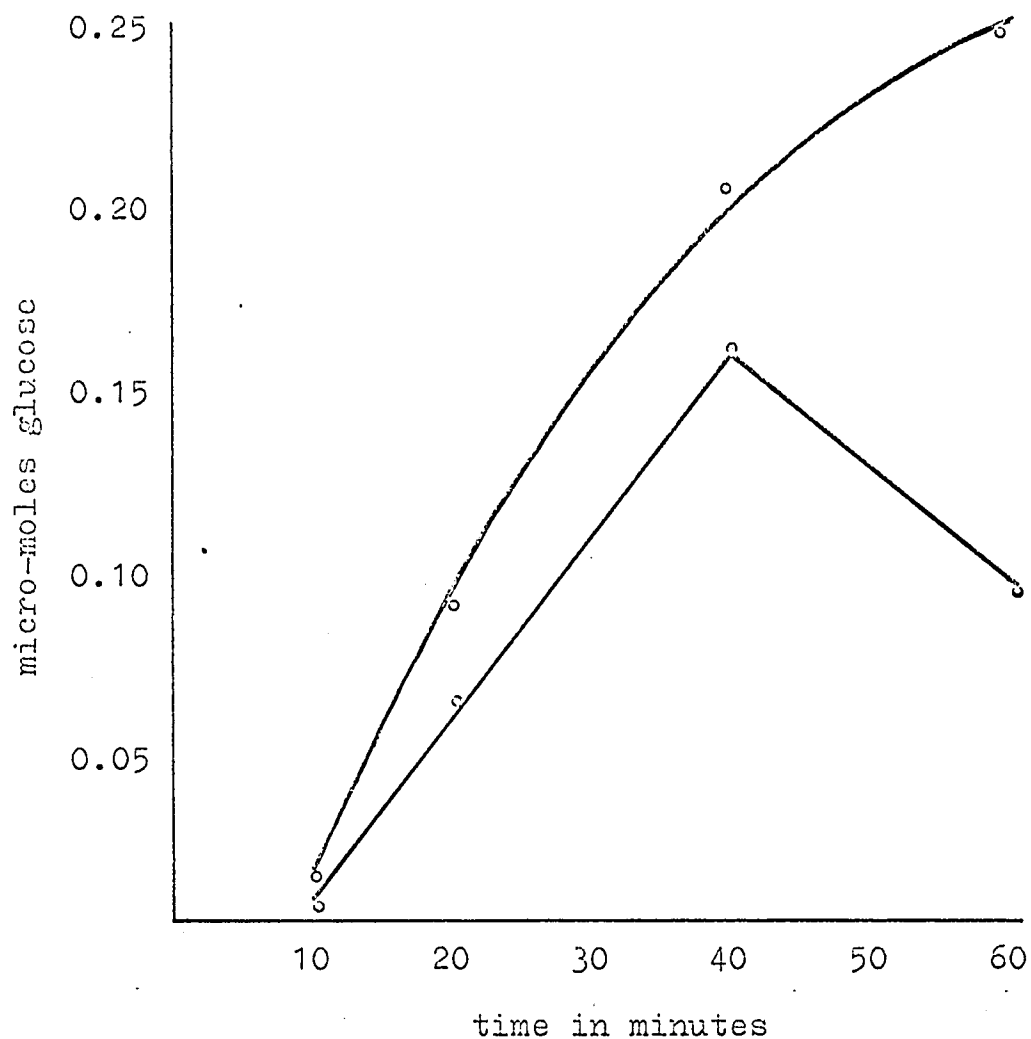
FIGURE 9.   GLUCOSE ^{14}C TRANSPORT VERSUS DRY OR WET WEIGHT

A paired comparison of the micro-moles of glucose trans-
port into the serosal fluid for T. mossambica versus time
calculated in micro-moles glucose/0.1 g wet weight (○)
and micro-moles glucose/0.1 g dry weight (●) (0.2 g/L mu-
cosal glucose).

glucose ^{14}C transport into everted gut segments of tilapia
the data for the sixty minute sample decreases from the
previous forty minute sample and is approximately 56% of
the expected value. This variation is typical of the va-
riation encountered by the author in trying to use dry
weight of gut segments to quantify data. It is interesting
to note that the data of Mussachia et al (1966) also exhib-
its wide variation and was likewise calculated in terms of
dry weight.

The probable explanation for wet weight rather than
dry weight of gut tissue being related to glucose transport
is that water absorption during incubation is related to
glucose transport, which is in turn related to the metabolic
state of the tissue. This is similar to the results of
Barry et al (1965) who found that fluid volume uptake was
related to the total solutes, such as glucose, transported.
Since dry weight does not seem to be related to the meta-
bolic state of the tissue and variations in dry weight be-
tween segments are very small, the variations in wet weight
of similar sized gut segments following incubation are an
indication of the amount of glucose transport that has
occurred.

In trying to quantify data in terms of gut segment
length great difficulty was encountered in getting precise
estimates of length due to stretching and contraction of
the tissue during eversion and the subsequent incubation.

No significant linear regression was found when the micro-moles of glucose ^{14}C transported into the everted gut sac of tilapia was plotted versus length in centimeters of the gut in Figure 10.

There was a relationship between wet weight and segment length in Figure 11 plotting length versus wet weight where P=0.025. The std. dev. was $\pm$ 0.1028 g/cm which would equal 0.05 micro-moles/0.1 mg wet weight/hour or about 25% of the average amount of glucose ^{14}C transported per hour as seen in Figure 12.

The rate of glucose ^{14}C transport into the serosal sac of tilapia is shown in Figure 12. The micro-moles of glucose/0.1 g wet weight of tissue are plotted versus time, and an analysis of linear regression, using Snedecor's model IA (1956), indicates that the standard deviation, sigma, is proportional to time. This indicates that the standard deviation, a reflection of the magnitude of variation, is a function of different rates of glucose transport in different fish, which is in general agreement with the idea of glucose transport being dependent upon one or more enzymatic reactions at some point and is consistent with the hypothesis put foreward earlier that different levels of endogenous glucose affect the rate of glucose transport. The regression of glucose transport on time was statistically significant (P=0.001).
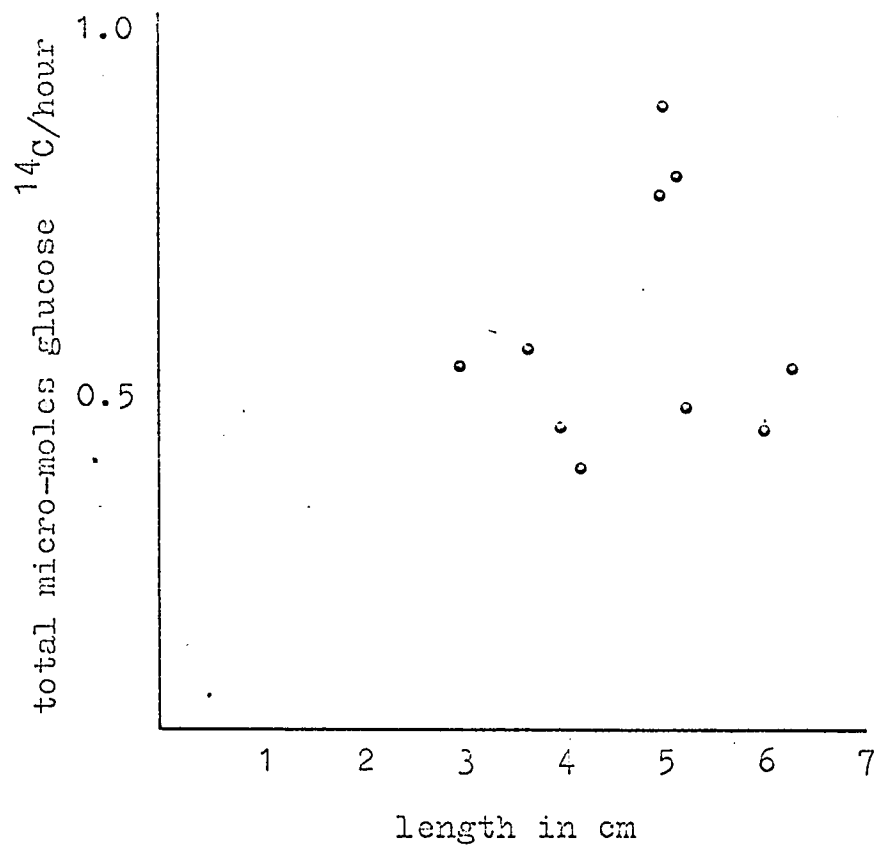
FIGURE 10. GLUCOSE ^{14}C TRANSPORT VERSUS LENGTH

Total micro-moles glucose ^{14}C transported into
the serosal fluid versus length of the gut sac
in centimeters for T. mossambica (0.2 g/L in
mucosal fluid).
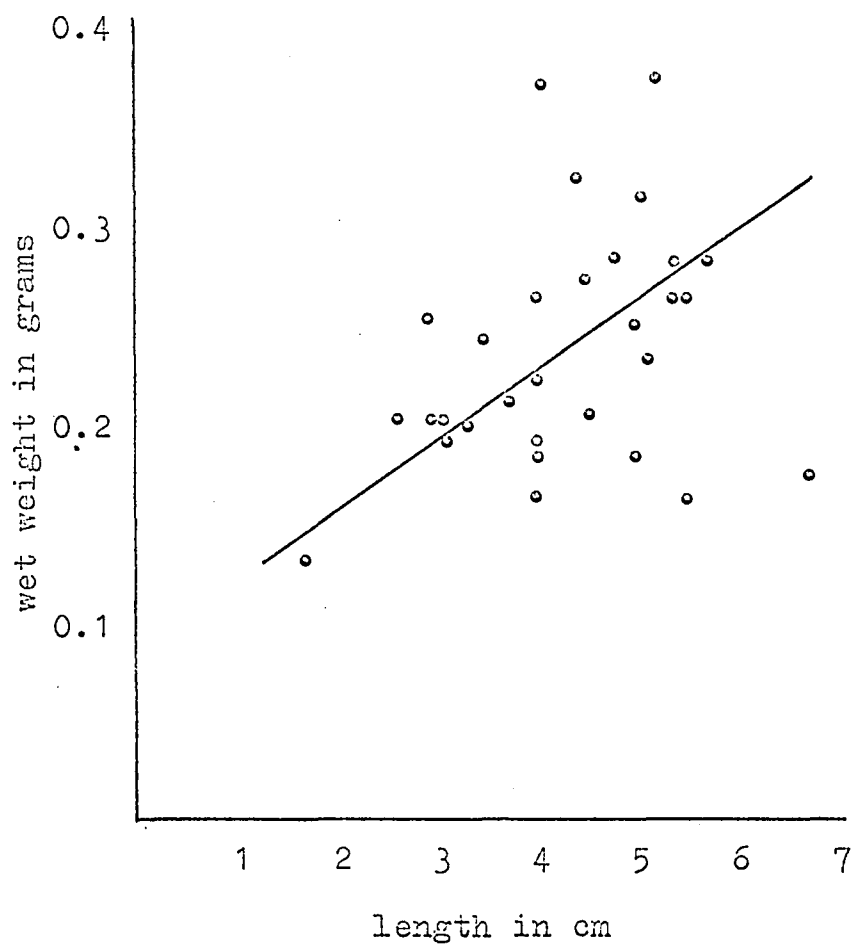
FIGURE 11. WET WEIGHT VERSUS GUT LENGTH

The relationship of wet weight to length
in everted gut segments for T. mossambica.
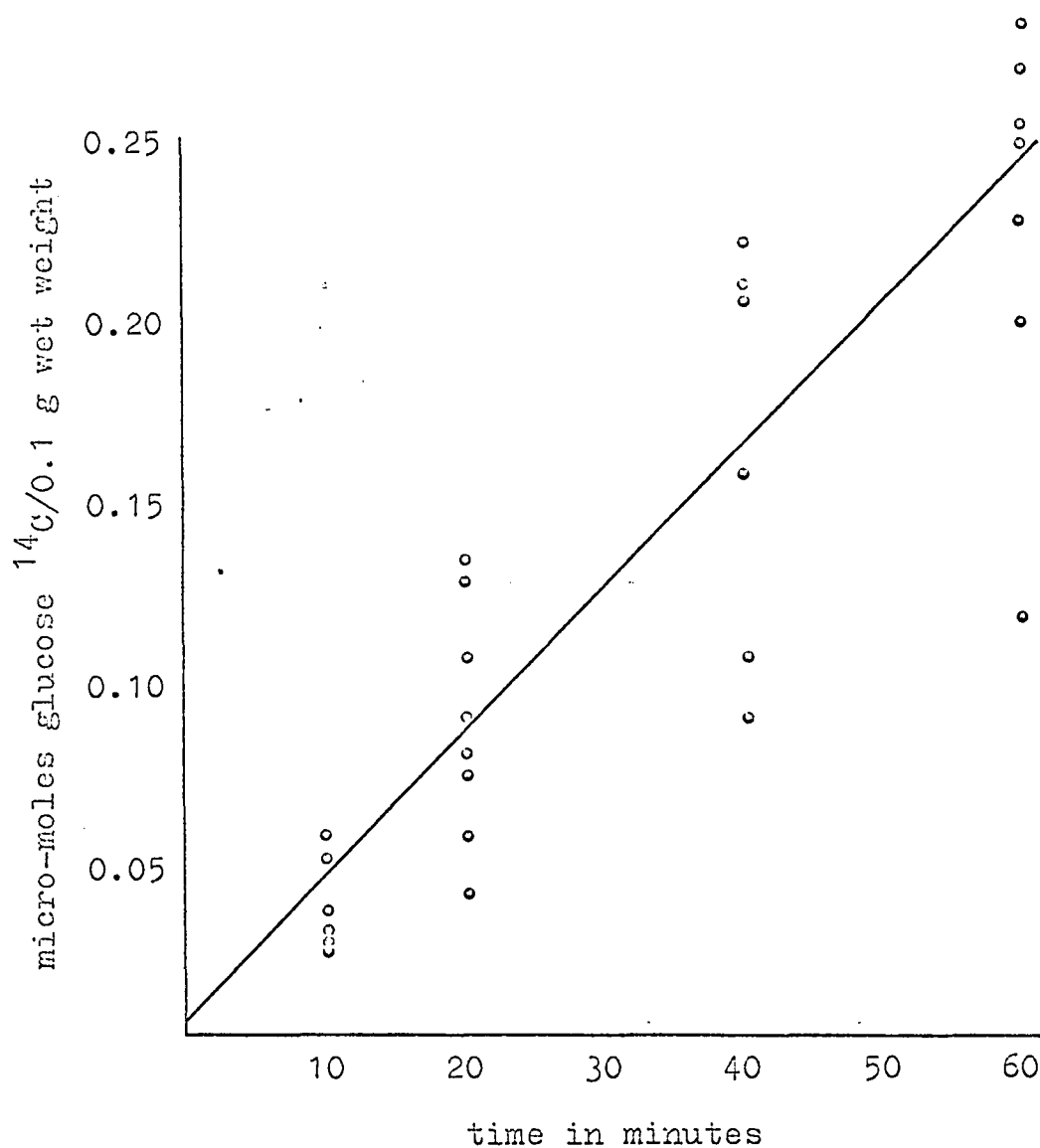
FIGURE 12. GLUCOSE ^{14}C TRANSPORT VERSUS TIME

Micro-moles glucose ^{14}C transported into the serosal fluid of T. mossambica versus time (0.2 g/L mucosal fluid glucose).

42.

The rate of uptake of glucose ^{14}C, as determined by analysis for 80% alcohol soluble tissue glucose, is shown in Figure 13. This data gives the same picture as the rate of glucose transport into the serosal sac shown in Figure 12. Again, the standard deviation, sigma, is proportional to time and indicates that accumulation of tissue glucose is a function of differing enzymatic rates. This is doubly important as it indicates that the everted gut segments do not take up all the glucose ^{14}C that they will transport into the everted sac as soon as they are put into the incubation medium, which would result in a high initial level of tissue glucose that gradually diffuses into the serosal sac.

Crane and Mandelstam (1960) found that the rate of tissue uptake by small rings of everted tissue was a more reproducable system than everted sac preparations in hamster guts with a standard deviation of $\pm$ 2.1 mm/ml for a fifteen minute preparation. For a ten minute preparation the standard deviation was $\pm$ 1.2 mm/ml indicating that he too found an increasing standard deviation with time for sugar uptake. In these experiments with tilapia the methods are not directly comparable, but the rate of uptake by tissue had a higher standard deviation, 0.07 micro-moles glucose/0.1 g wet weight of tissue/hour, than the serosal fluid determination, which had a standard deviation of 0.04 micromoles glucose/0.1 g wet weight of tissue/hour. This may
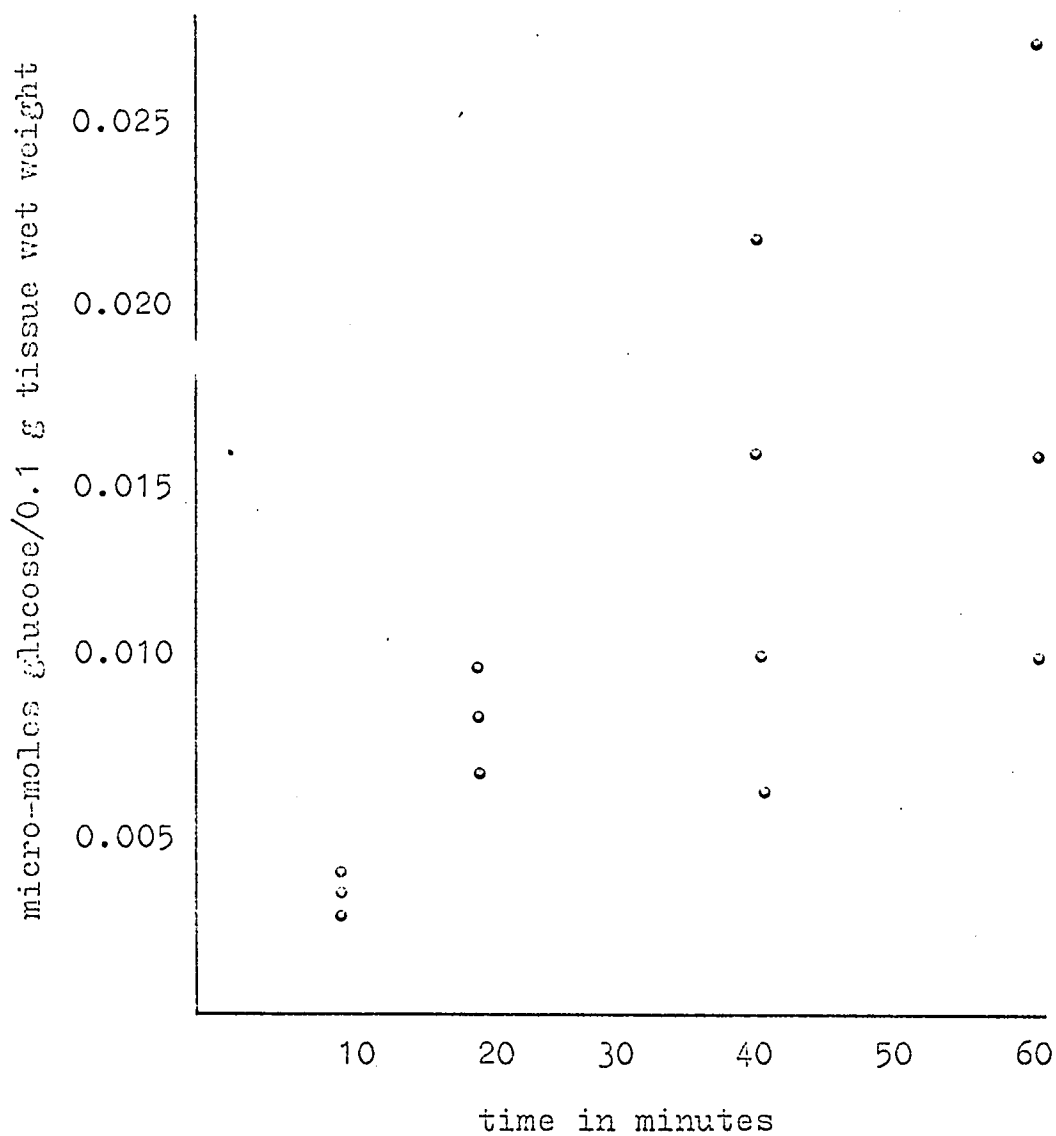
FIGURE 13. TISSUE GLUCOSE ^{14}C VERSUS TIME

Micro-moles of glucose ^{14}C extracted from everted gut sacs of T. mossambica with 80% ethyl alcohol after varying time periods of incubation in 0.2 g/L mucosal glucose.

possibly be due to the increased number of manipulations required to measure glucose through paper chromatography. Crane and Mandelstam (1960) point out that kinetic studies are best performed using tissues of the same potential activity. The purpose of quantifying the data is to reduce variability. If the method used to quantify the data does not in some way account for the variability in preparations due to the rate limiting step in the carrier process, the percentage of the variability will either stay the same or increase.

In the case of tilapia it appears that wet weight of tissue is the best method of quantifying the data but the author acknowledges that a better way would be to quantify the data in terms of the rate limiting step in the carrier process, if that were possible.

EXPERIMENTAL METHODS AND GLUCOSE TRANSPORT: "All that glitters is not gold" (anon.), and all the ^{14}c activity that passes into the serosal sac is not in the form of glucose ^{14}C. In Figure 14 the total ^{14}C and glucose ^{14}C (as determined by paper chromatography) calculated in micro-moles glucose/0.1 g wet weight of gut segments is plotted versus time for eight fish. In Table II the amounts of total ^{14}C and glucose $^{14}C/0.1$ g/hour and the percentages are shown. Glucose ^{14}C averaged 88% of the total ^{14}C activity. It seems probable that most of this difference (about 12% at sixty minutes) is due to metabolism.
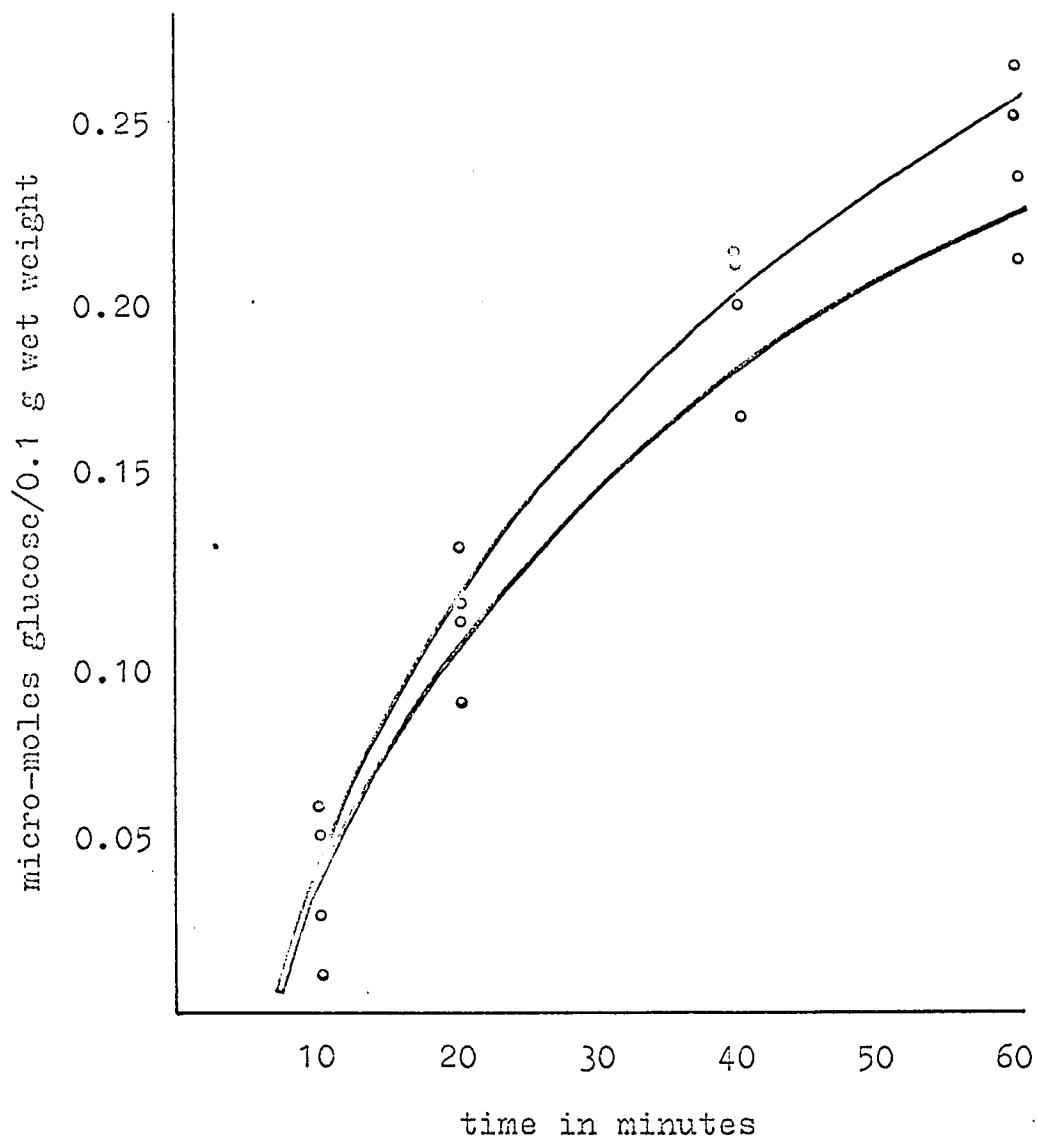
FIGURE 14. TOTAL ^{14}C AND GLUCOSE ^{14}C TRANSPORT VERSUS TIME

Total ^{14}C (●) and ^{14}C glucose (○) transported into the serosal sac versus time for T. mossambica (0.2 g/L mucosal glucose).

TABLE II

TOTAL ^{14}C AND GLUCOSE ^{14}C

A comparison between the amount of glucose ^{14}C and total ^{14}C activity in the serosal fluid of T. mossambica expressed as micro-moles glucose ^{14}C/0.1 g wet weight/hour.

fish	total ^{14}C	^{14}C glucose	per cent
1	0.250	0.21	84
2	0.26	0.24	91
3	0.23	0.22	97
4	0.23	0.19	83
5	0.23	0.19	83
6	0.24	0.22	<u>93</u>
			88

This is similar to, but not as pronounced as, the results of Mussachia et al (1966) who found that 45 minute incubation periods were more reliable estimates of transport, as after sixty minutes "there was an obvious inconsistency of results." Carlisky and Huang (1962) did not demonstrate active transport into the serosal fluid, although this was perhaps an artifact due to the large (10 ml) serosal volume.

In Table III the effect of time on the transport of glucose into the serosal sac is shown for A. sandvicensis taken from paired data using the method of Jorgenson et al (1961).

The difference of 0.53 micro-moles/ ml glucose between the first and the second forty minute period is significant at the 0.05 probability level (P=0.005, d.f=4) according to the paired comparison method of Snedecor (1956). The difference is probably associated with changes in metabolic activity in the cells, since controls run at the same time indicated that the difference was not due to endogenous glucose.

The rate of glucose ^{14}C incorporation into glycogen is shown in Figure 15 where micro-moles of glucose are plotted versus time. Approximately 1.100 as much glucose is incorporated into 10% TCA soluble glycogen as is transported into the serosal fluid.

From the preceding results and discussion it should be clear that the transport of glucose across the everted

TABLE III

DECREASE IN GLUCOSE TRANSPORT ABILITY WITH TIME

The decrease in transport of reducing glucose in meq/L into the serosal sac of A. sandvicensis in paired sacs for two successive 40 minute time periods.

fish	time 1	time 2	difference
1	5.66	4.72	−0.94
2	4.41	4.05	−0.36
3	4.77	4.16	−0.61
4	4.33	3.88	−0.45
5	4.83	4.27	−0.56
			−0.58
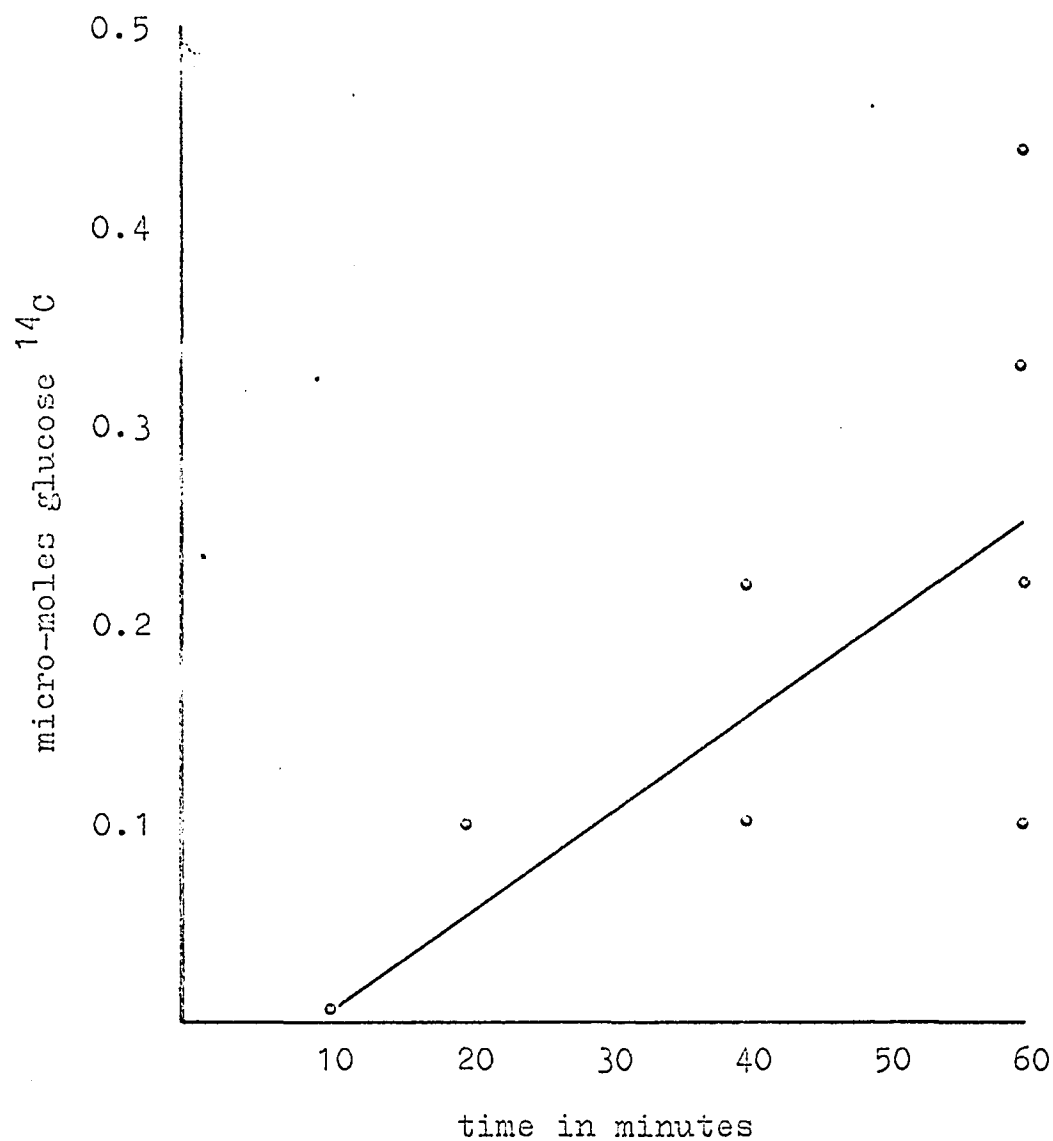
FIGURE 15.   TISSUE GLYCOGEN ^{14}C VERSUS TIME

Formation of glycogen during transport of glucose ^{14}C across the everted gut of T. mossambica expressed as micro-moles glucose per hour.

intestinal sac preparation in fish is a complex subject with many potential variables inherent in the experimental method. A major source of variability between different fish appears to be due to the level of endogenous glucose in the tissue and its presumed effect on intracellular metabolism. The possibility that variations in the level of enzyme or carrier molecule can cause changes in the rate of glucose transport is studied in the following chapters.

IV. ENVIRONMENT AND DIET

GLUCOSE TRANSPORT AND ENVIRONMENT: The movement of glucose
into the everted intestinal segments of tilapia is the same
for fish that have been raised in fresh or salt water, as
can be seen from Figures 16 and 17, where reducing sugar
glucose and glucose ^{14}C in the serosal fluid are plotted
against time. A statistical analysis of co-variance using
the method of Snedecor (1956) indicates that there is no
significant difference between the regressions in Figure 16
and that the differences are due to random variation be-
tween fish (for slope F = 1.21, d.f. = 1.21, P = more than
0.05; for means F = 2.36, d.f. = 1.21, P = more than 0.05).
Similarly, there is no significant difference between the
regressions in Figure 17.

The differences in glucose transport in fresh and salt
water fish that other authors, such as Mussachia et al
(1961, 1962, and 1963) and Wilson (1957) found, have been
shown to be due to aereation of the mucosal fluid with air
instead of oxygen by Mussachia et al (1966). The inability
of the flounder to transport glucose, as reported by Rout
et al (1965), is not so easy to explain, as 95% oxygen was
used in their experiments. However, in their experiments
they used a sea water based Ringers, and this excess amount
of sodium compared to the normal blood and intestinal sodi-
um levels for marine teleosts may be the cause of their
failure to demonstrate glucose transport.
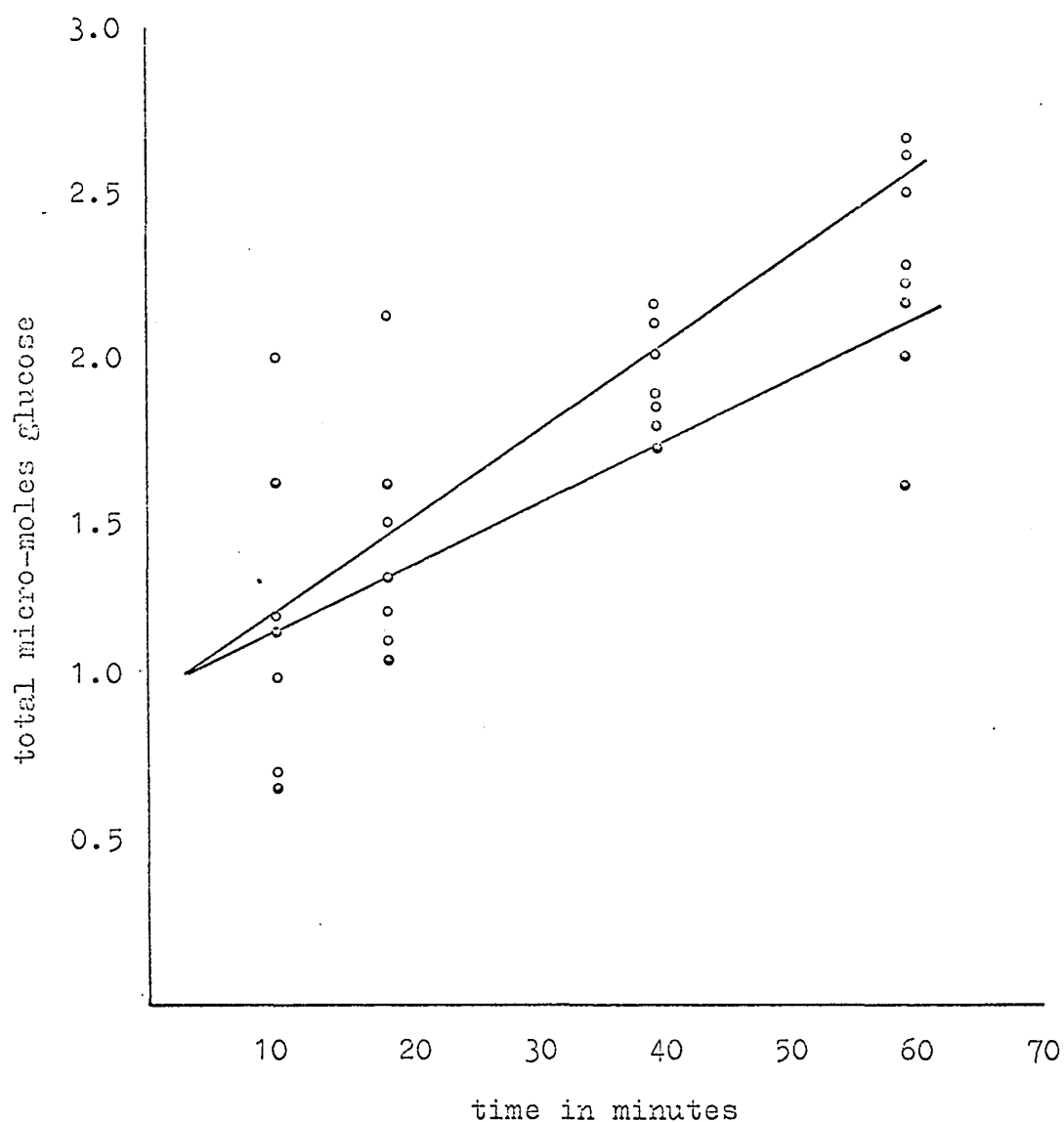
FIGURE 16.   ENVIRONMENT AND GLUCOSE TRANSPORT

Micro-moles of glucose (in reducing equiva-
lents) transported into the serosal fluid of
T. mossambica versus time for fish raised in
fresh water (○) and salt water (•) (1.0 g/L
glucose in mucosal fluid; 1.0 ml serosal
fluid).
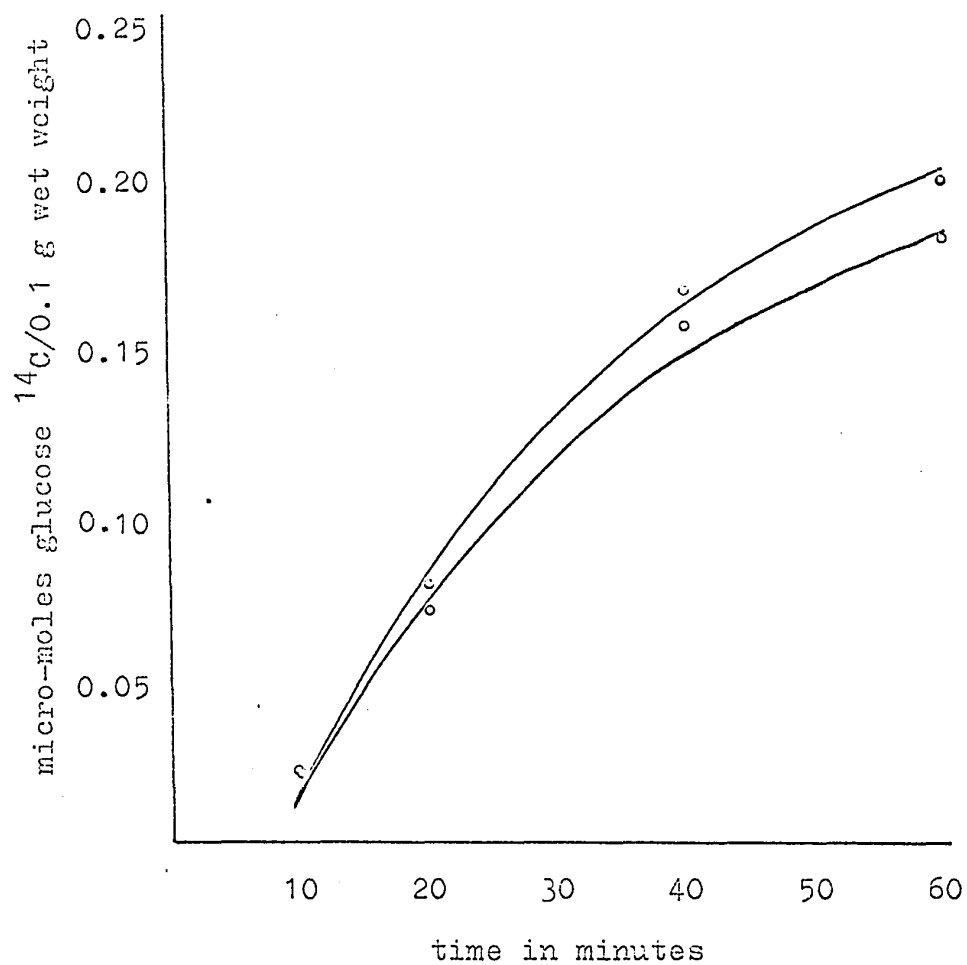
FIGURE 17.   ENVIRONMENT AND GLUCOSE ^{14}C TRANSPORT

Micro-moles glucose ^{14}C transported into the ser-
osal fluid versus time for T. mossambica raised
in fresh water (○) and salt water (○) (0.2 g/L
mucosal glucose). The numbers in parenthesis
indicate the number of fish for each point.

A comparison of the rates of absorption for tilapia raised in salt water and for the marine surgeonfish A. sandvicensis, as seen in Figure 18, indicates that glucose ^{14}C is transported twice as fast in the surgeonfish as in tilapia. Microscopic examination of the intestine of both fish indicates that the surgeonfish has innumerable villi throughout the length of the intestine but that tilapia has longitudinal folds.

Regardless of the reason for the difference in glucose transport between the surgeonfish and tilapia, it is clear that the rate of transport for fish raised in fresh water or salt water is due to the species of fish used, not to the environment.

SODIUM TRANSPORT AND ENVIRONMENT: Raising tilapia in fresh or salt water was similarly found not to affect the rate of sodium transport into the serosal fluid of the everted gut segments. Figure 19, which plots the rate of transport of ^{22}Na for fresh or salt water raised fish versus time, indicates that the rates are about the same, and an analysis of co-variance using the method of Snedecor (1956) shows that there is no statistically significant difference between the two. The presence or absence of glucose in the mucosal fluid likewise did not cause a significant difference in sodium transport.

The data for the transport of ^{22}Na into the serosal sac was calculated in terms of wet weight of tissue, as was
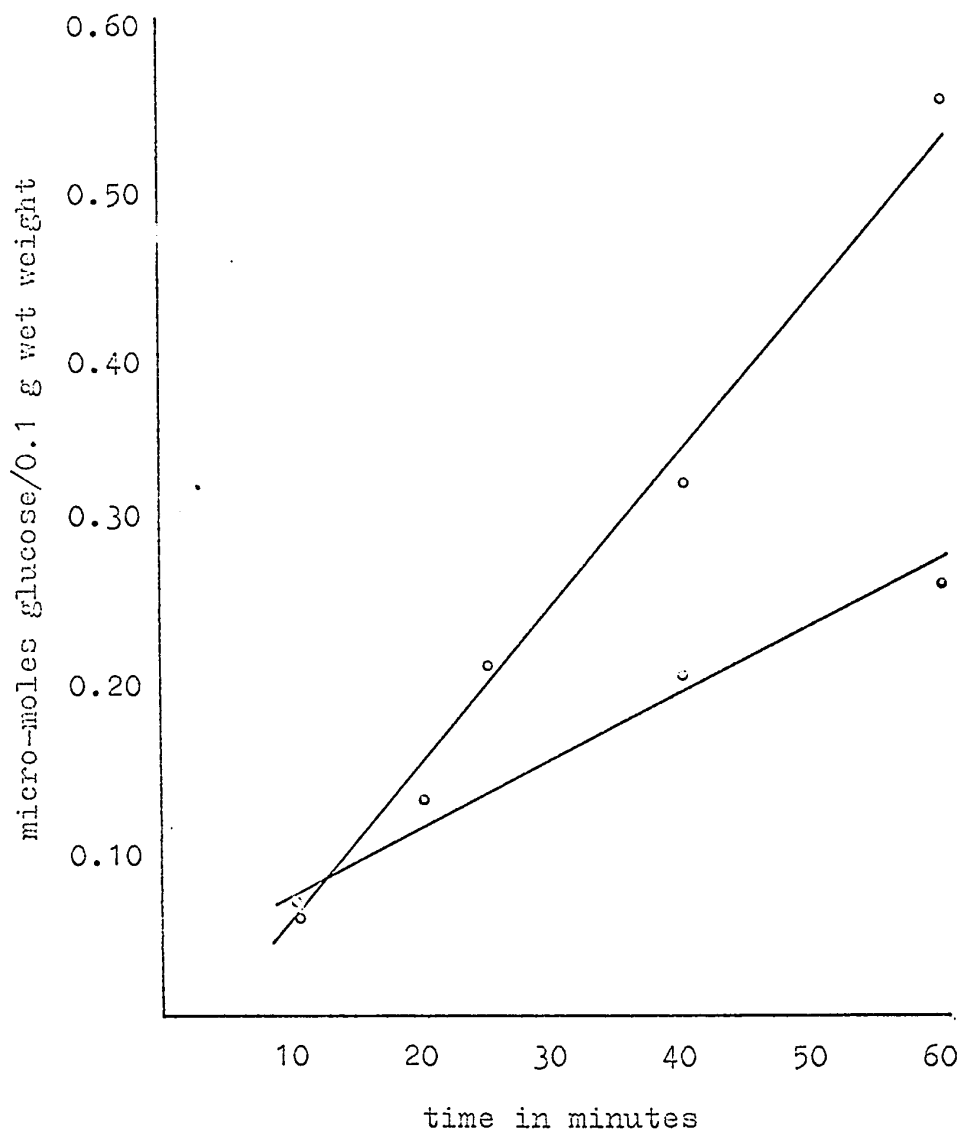
FIGURE 18. GLUCOSE TRANSPORT FOR A. SANDVICENSIS

VERSUS T. MOSSAMBICA

Transport of glucose ^{14}C into the serosal fluid of A. sandvicensis (o) and T. mossambica (o) (0.2 g/L glucose in the mucosal fluid).
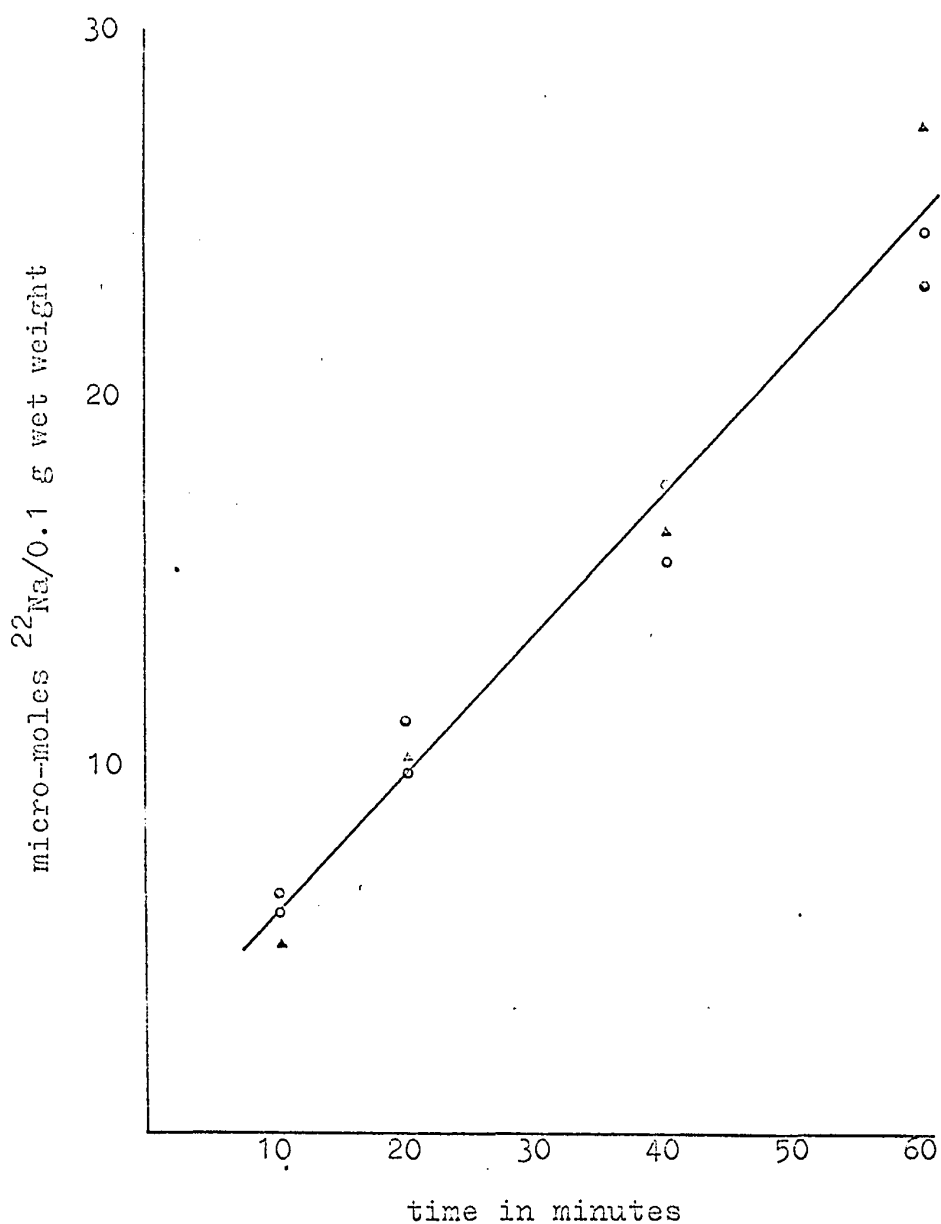
FIGURE 19.   SODIUM TRANSPORT VERSUS ENVIRONMENT

Transfer of sodium into the serosal fluid of T.
mossambica raised in fresh water (o) and salt
water (▲o) with 0.2 g/L glucose (▲o) and no glu-
cose (o) in the mucosal fluid. Each point is a
mean for 4 fish.

done with glucose ^{14}C transport. In Figure 20 the regression of ^{22}Na on wet weight of tissue is presented. The regression is significant: P=0.02, d.f. 8.

The change in sodium level of the serosal fluid during the course of glucose transport into the serosal sac was investigated using flame spectrophotometry. The results, shown in Figure 21, indicate that there is a slight loss of sodium from the serosal fluid, but a statistical analysis of regression using the method of Snedecor (1956) indicates that this apparent loss is due to random variation between fish and is not statistically significant.

The sodium level of the blood and intestinal fluid of tilapia raised in both fresh and salt water and of A. sandvicensis was determined in vivo using flame spectrophotometry. The mean sodium level was found to be slightly higher for the intestinal fluid than the blood in all cases, as can be seen in Table IV. Whether this is a reflection of the fact that the blood was collected from the heart and not from the intestinal area is not known. The everted intestinal segments also showed a slight tendency to lose sodium, which was, however, not statistically significant.

Both House and Green (1963) and Smith (1964) have demonstrated a net sodium transfer in vitro from the mucosal to the serosal fluid. Their periods of incubation were 260 minutes instead of the sixty minute periods used in this experiment.
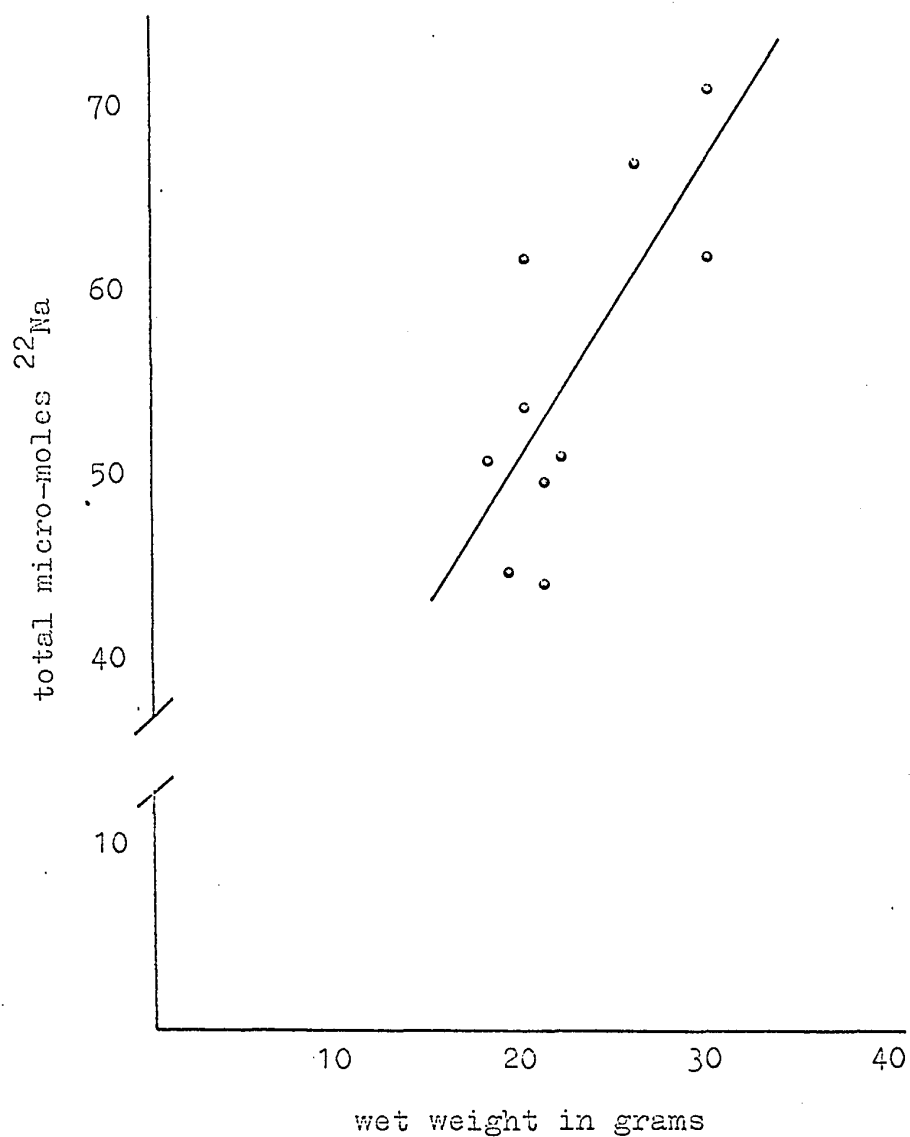
FIGURE 20. SODIUM TRANSPORT VERSUS WET WEIGHT

Transport of ^{22}Na into the serosal fluid versus wet weight of the everted sacs in grams for $\underline{T}$. $\underline{mossambica}$.
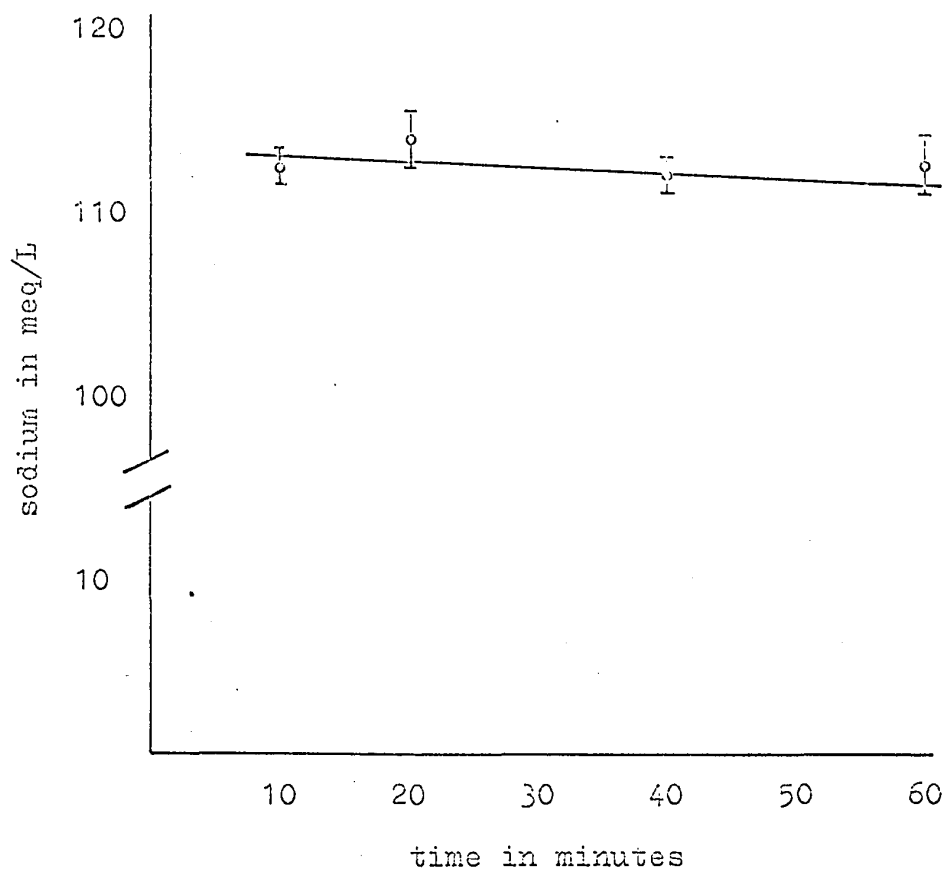
FIGURE 21. SODIUM CONCENTRATION IN VITRO VERSUS TIME

Fluctuation of sodium in the serosal fluid of T. moss-
ambica versus time in everted gut segments incubated
in 0.2 g/L mucosal fluid glucose (each point is the
mean of 4 fish).

TABLE IV

SODIUM CONCENTRATION IN VIVO

The blood serum (Bld) and intestinal fluid (Int) sodium concentration for A. sandvicensis (A) and for T. mossambica raised in fresh water (B) and salt water (C) as expressed in meq/L.

A	Bld	163.7	169.0	177.5	186.2	205.2
	Int	171.1	186.2	193.3	165.5	198.3
B	Bld	152.0	149.0	155.5	145.7	152.0
	Int	108.0	154.5	164.4	160.0	166.6
C	Bld	127.0	153.8	145.5	148.9	153.8
	Int	163.5	166.6	165.0	161.7	149.0

The osmotic pressure of the blood and intestinal fluid of <u>A</u>. <u>sandvicensis</u> was determined <u>in vivo</u> using a Fiske osmometer. The results, summarized in Table V, show that there is an average of 24.5 milliosmoles greater osmotic pressure in the intestine than in the blood. This difference may be an artifact due to the removal of serum protein by clotting.

The osmotic pressure in the serosal and mucosal fluids in everted sac preparations of <u>A</u>. <u>sandvicensis</u> after one hour incubation was determined for preparations which ac= tively transported glucose. The results, shown in Table VI indicate that the serosal fluid is hypertonic to the mucosal fluid, with a mean for the difference of 9 ($\pm$ 4) milliosmoles. The results of the <u>in vitro</u> experiments are opposite those of the <u>in vivo</u> flame spectrophotometry measurements, so the increase in osmotic pressure is probably not due to sodium ion. Control experiments with an equivalent amount of glucose added to the Ringers solution did not result in similar increase in osmotic pressure, so the increase cannot be due to the movement of glucose. The increase in serosal osmotic pressure is probably an artifact of the small serosal volume and the liberation of proteins from the serosal membrane and ruptured veins and arteries. <u>GLUCOSE TRANSPORT AND DIET</u>: Feeding galactose (one gram per fish per day) in the diet did not affect the rate of glucose or glucose ^{14}C transport into the everted gut

TABLE V

OSMOTIC PRESSURE IN VIVO

The difference in osmotic pressure in milliosmoles between the intestine and the blood serum in A. sandvicensis.

Intestine	Blood	Difference
398	415	-17
507	438	+69
488	468	+20
392	412	-20
500	440	+60
410	384	+20
420	386	+34
		+24.5

TABLE VI

OSMOTIC PRESSURE IN VITRO

The difference in osmotic pressure in milliosmoles/hour between the serosal and mucosal fluids of A. sandvicensis in everted sac preparations that actively transported glucose.

Serosal	Mucosal	Difference
378	370	+8
380	371	+9
377	370	+7
382	372	+10
381	372	+9

$$\bar{d} = 9$$

segments as can be seen in Table VII. There is no significant difference between the means of the control groups and the groups fed galactose.

It is difficult to compare these results with those of Westenbrink (1936) who showed a 50 to 100 per cent increase in glucose or galactose transport in rats fed a five per cent solution of galactose in their water because different animals and experimental procedures are involved.

However, Diedrich and Anderson (1960) found that rats fed eight per cent galactose or glucose in their diet did not accumulate galactose 1-PO_4 faster than the control group, likewise indicating that no induced enzymatic synthesis occurred.

Westenbrink's results indicate that feeding galactose to the rats resulted in induced enzyme formation that accelerated glucose and galactose transport. In tilapia neither changes in diet nor environment affected glucose transport and therefore it may be assumed that an inducible enzyme system related to glucose transport does not exist in fish. Since the magnitude of variation in glucose transport in tilapia has already been shown to be within the level of variation experimentally induced by the addition of endogenous glucose, one must assume that the level of the glucose carrier remains relatively constant from fish to fish for the same area of the intestine.

TABLE VII

GLUCOSE TRANSPORT AND DIET

The transport of glucose into the serosal fluid of the everted gut sac in T. mossambica fed galactose (F) and trout pellets (C).

^{14}C glucose (micro-moles glucose/0.1 g wet weight)

						average
F	0.25	0.21	0.26	0.25	0.21	0.24
C	0.23	0.24	0.23	0.24	0.24	0.23

Reducing glucose (micro-moles glucose/ml)

					average
F	0.94	0.78	1.28	1.42	1.11
C	0.83	0.94	1.42	1.56	1.17

V. THE RELATION BETWEEN GLUCOSE
AND SODIUM TRANSPORT

THE SODIUM REQUIREMENT FOR GLUCOSE TRANSPORT: The trans-
port of glucose into the serosal fluid of the everted in-
testinal sac of tilapia is dependent on the sodium con-
centration of the Ringers solution that bathes the sac, as
can be seen in Figure 22. The transport of glucose ^{14}C is
significantly less for experiments where the sodium level
is below 100 meq/L. This effect is not due to a simple
change in osmotic pressure of the sodium because in half of
the experiments the solutions were kept isoosmotic by the
addition of sucrose without changing the results.

A similar dependence of glucose transport on sodium
concentration has been reported for rabbits by Schultz and
Zalusky (1964), hamsters by Bihler and Crane (1962), guinea
pigs, rats, and hamsters by Lyon and Crane (1966), and for
the toad by Csaky and Thale (1960).

It is clear that tilapia, like other animals, require
sodium ion for glucose transport.

The sodium level in the blood serum and intestinal
fluid of tilapia and A. sandvicensis in vivo can be seen in
Table IV. The in vivo blood and intestinal sodium level is
higher than the minimum level, 100 meq/L, required for max-
imal glucose transport.

GLUCOSE EFFLUX AND SODIUM: The efflux of endogenous glu-
cose, as measured by reducing value, into the serosal fluid
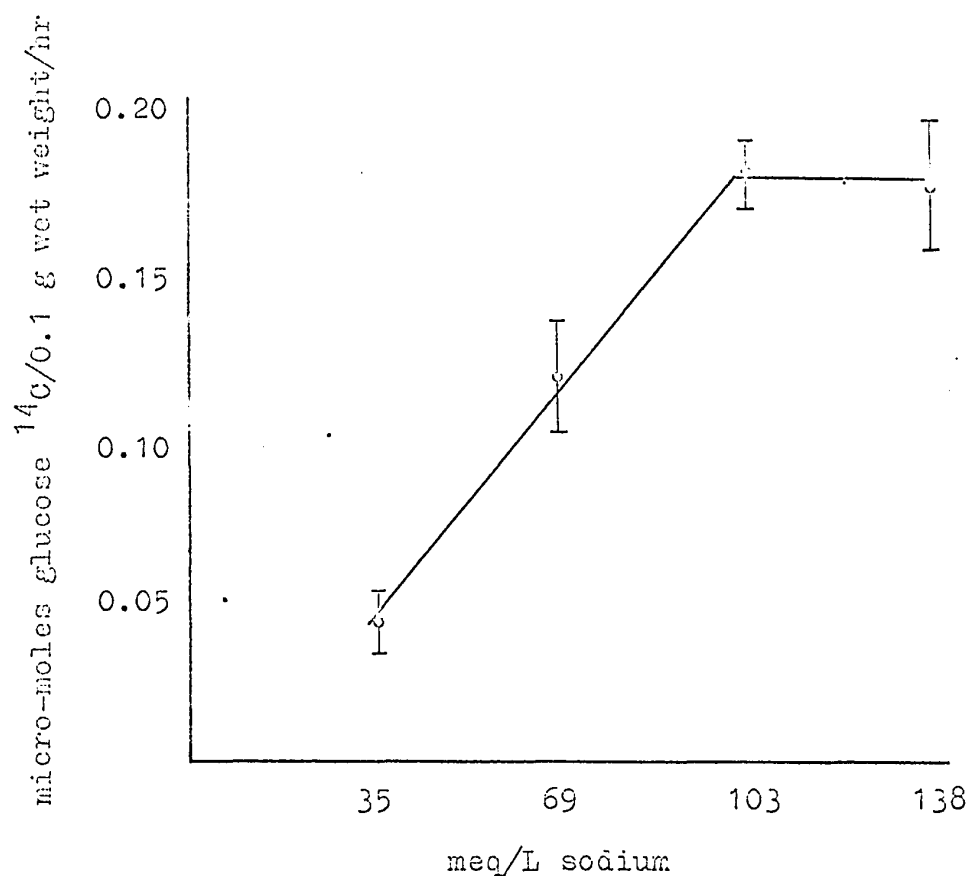
FIGURE 22. GLUCOSE TRANSPORT VERSUS SODIUM CONCENTRATION

Glucose ^{14}C transport into the serosal fluid of T. moss-
ambica versus sodium concentration of the serosal and mu-
cosal fluids (0.2 g/L glucose in the mucosal fluid).

in experiments where there was less than 100 meq/L sodium

in the Ringers solution can be seen in Figure 23. This is

similar to the results of Crane (1965) who found outflow of

6-deoxy-glucose from the intestinal epithelium of rats

under similar conditions. He took this to indicate that

glucose transport and the sodium pump were intimately re-

lated and that by changing the direction of the sodium gra-

dient the direction of glucose transport could be reversed.

In this experiment the efflux of glucose is signifi-

cant at 69 meq/L, before the sodium gradient between the in-

side of the cell and the medium is reversed. There can be

no question that glucose transport is very sensitive to so-

dium ion concentration, but these results would seem to

contradict the idea that the efflux of glucose is due to a

reversal of the sodium gradient and that the movement of

glucose depends upon the sodium gradient for energy.

The theories of glucose transport involving sodium put

forth by Crane (1962 and 1965) and Crane et al (1961 and

1965) involve a mobile carrier molecule that binds to both

sodium and glucose and shuttles back and forth across the

membrane. Because glucose outflow, as measured by reducing

value, and glucose transport, as measured by accumulation

of glucose ^{14}C in the serosal fluid, occur at the same time

in this experiment, glucose transport would have to be a

function of different rates of transport in two directions.

Absolute dissociation of the sodium and glucose at one mem-

brane surface did not occur. This means that the carrier
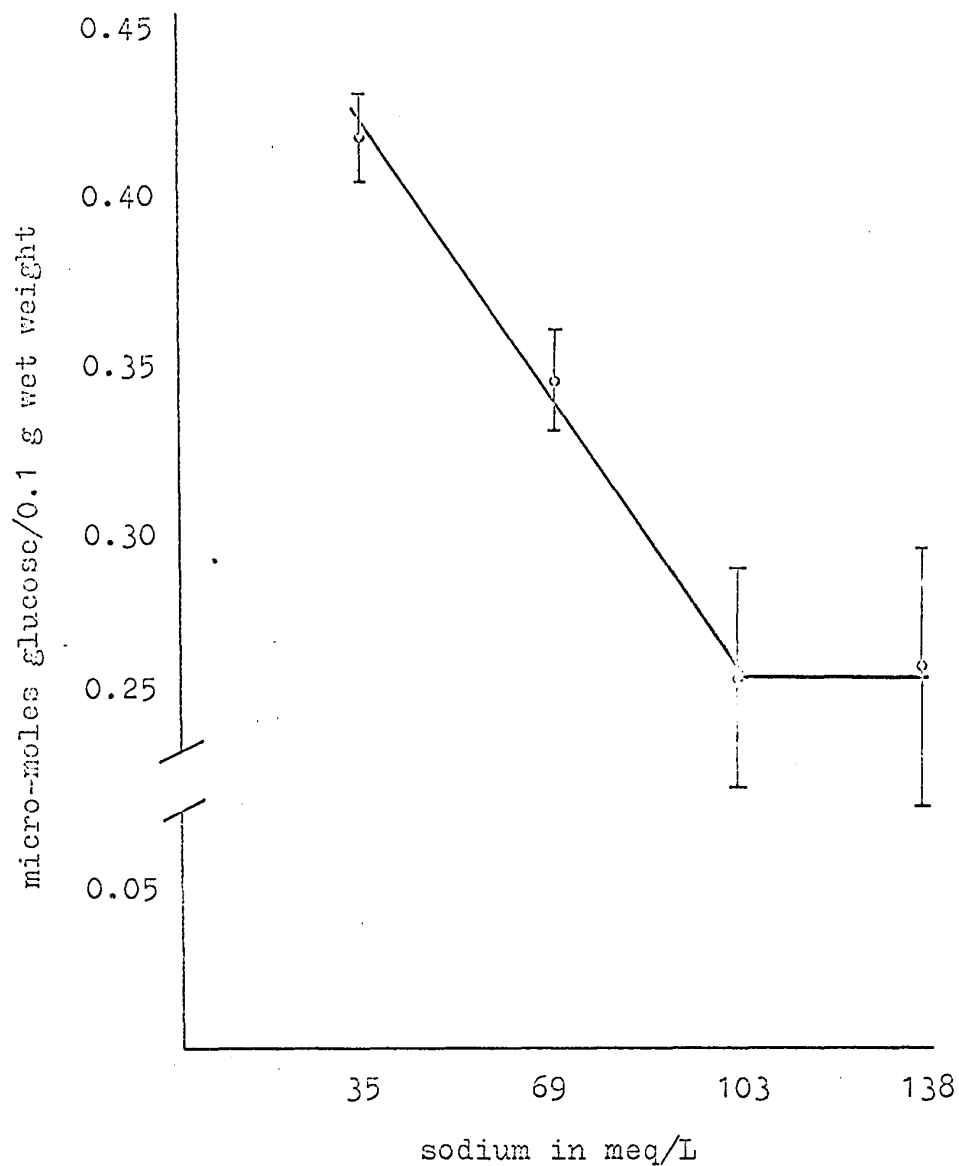
FIGURE 23.   GLUCOSE EFFLUX VERSUS SODIUM CONCENTRATION

Counter transport of endogenous glucose into the sero-
sal fluid versus sodium concentration of the incuba-
tion medium for the everted gut preparations for T.
mossambica (0.2 g/L glucose in mucosal fluid).

molecule could not be moving back across the membrane empty,
but that some carrier molecules would have to move back
loaded with glucose and sodium. This in itself eliminates
the possibility that the energy for glucose transport comes
from a simple electrical field phenomena due to the sodium
gradient between the cell and the medium. How could a
loaded carrier move against an electric field if that field
is to supply the energy for transport?

This is similar to the conclusions drawn by Newey and
Smyth (1964) who conclude that in their experience in-
creased sodium transport in the presence of glucose would
require a sodium pump in the opposite direction to that re-
quired for glucose transport. In this case, the sodium pump
would have to be pumping in two directions at different
rates, creating two different electrical fields that the
carrier could differentiate between to transport glucose
both into and out of the cell at the same time.

AREAS OF TRANSPORT: Reinforcing the view that sodium and
glucose transport are not linked together are the results
from the study of glucose ^{14}C and ^{22}Na transport in the
different areas of the intestine of tilapia. As can be seen
in Tables VIII and IX the transport of sodium into the sero-
sal sac either increases or stays the same from the ante-
rior regions of the intestine to the posterior, regardless
of whether the data is quantified using wet weight or
length. The transport of glucose, on the other hand,

TABLE VIII

AREAS OF THE GUT AND TRANSPORT

The transport of glucose ^{14}C and ^{22}Na into the serosal fluid of everted gut sacs of T. mossambica in micro-moles of glucose or sodium/0.1 grams wet weight for the stomach (St) and the pyloric (Py), upper (Up), middle (M), and lower (L) quarters of the intestine.

Glucose

fish	St	Py	Up	M	L
1	0	0.41	0.18	0.31	0.19
2	0.11	0.51	0.41	0.31	0.30
3	0	0.42	0.31	0.36	0.36
Average	0.03	0.45	0.30	0.32	0.28
Ouabain	0.07	0.08	0.13	0.21	0.13
KCN	0.02	0.08	0.10	0.20	0.06

Sodium

fish	St	Py	Up	M	L
1	0	2.75	6.89	12.75	11.20
2	0	16.03	48.10	47.58	37.58
3	0.06	10.34	31.37	35.68	30.57
Average	0.68	9.65	28.79	32.06	26.37
Ouabain	2.58	16.20	11.72	30.51	22.06
KCN	4.82	8.44	12.24	12.58	13.18

TABLE IX

AREAS OF THE GUT AND TRANSPORT BY LENGTH

The transport of glucose ^{14}C and ^{22}Na into the serosal fluid of everted gut sacs of T. mossambica in micro-moles of glucose or sodium/cm length for the stomach (St), and the pyloric (Py), upper (Up), middle (M), and lower (L) quarters of the intestine.

Glucose

fish	St	Py	Up	M	L
1	0	0.08	0.04	0.03	0.03
2	0.14	0.12	0.03	0.02	0.02
3	0	0.07	0.02	0.02	0.01
Average	0.05	0.09	0.03	0.02	0.02
Ouabain	0.07	0.01	0.01	0.01	0.01
KCN	0.04	0.03	0.02	0.03	0.01

Sodium

fish	St	Py	Up	M	L
1	0	0.6	1.5	1.5	1.7
2	0	3.8	3.3	2.8	2.6
3	2.8	1.7	1.8	2.4	2.0
Average	0.9	2.1	2.2	2.2	2.1
Ouabain	2.6	1.9	1.1	1.6	1.9
KCN	7.2	3.3	2.2	1.6	2.2

decreases from the anterior to the posterior regions of the intestine regardless of whether the data is quantified using wet weight or length of the segments. If glucose and sodium share the same carrier the transport of glucose and sodium should be of the same order of magnitude throughout the length of the gut.

In Figure 24 the micro-moles of sodium transported into the serosal fluid are plotted versus the length of the gut segments for three fish. It can be clearly seen that each of the fish transports sodium at a constant rate for that fish and that different fish transport sodium at different rates, since all the segments from each fish fall into distinctly different regressions. This indicates that sodium transport is related to length (surface area) and an unknown factor, presumably enzyme level, which is constant throughout the gut within a particular fish but which varies from fish to fish.

These results showing greatest transport at the pyloric end of the gut of tilapia are different from those of Wiseman (1954), using rats, Crane and Mandelstam (1960) using hamsters, and Stokes and Fromm (1964) using rainbow trout, who found that the most active area of absorbing glucose was in the middle region of the intestine, which would correspond to the upper and middle quarters in this experiment. All of these other results are based on different methods than those used in this study. Crane and
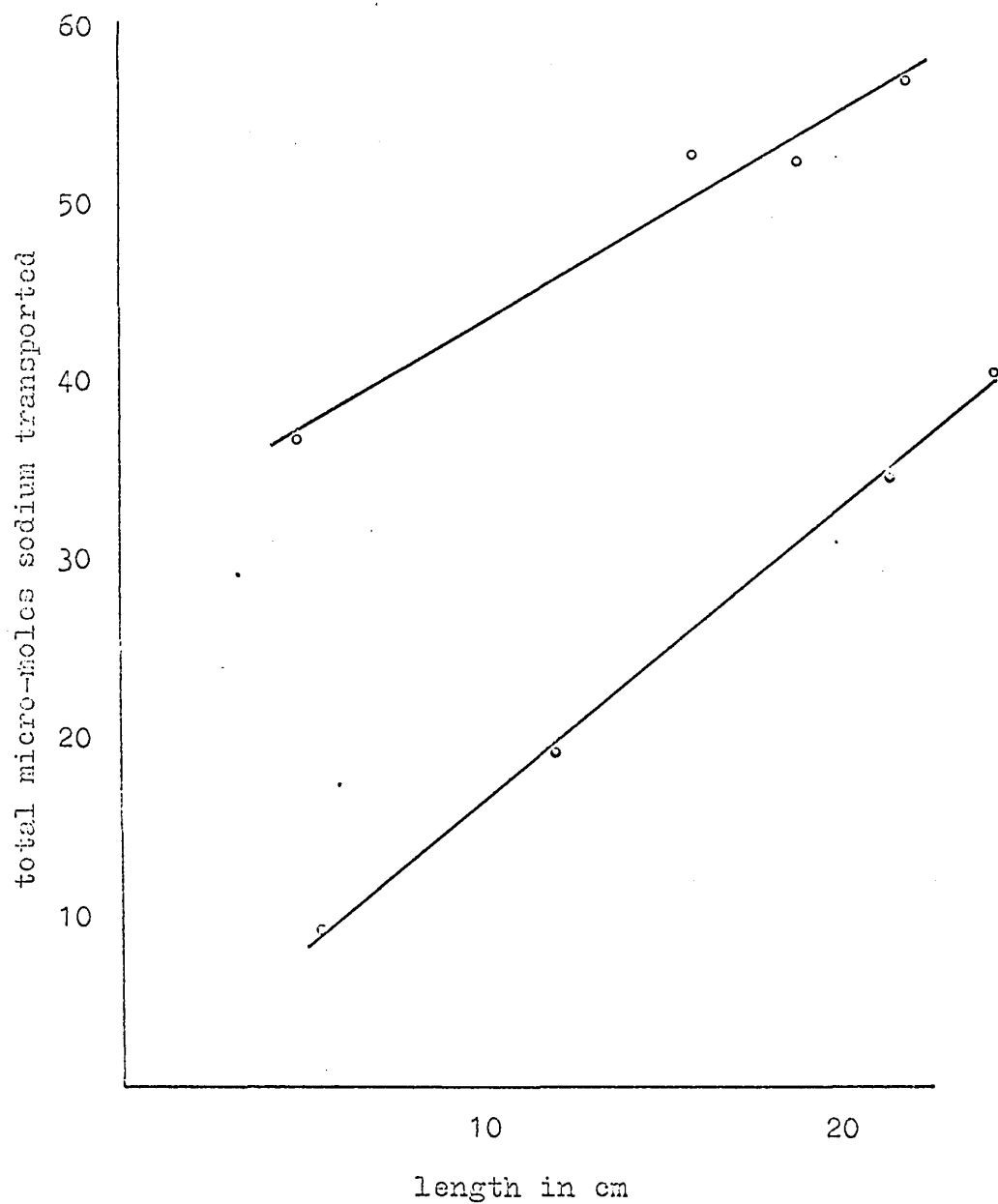
FIGURE 24. SODIUM TRANSPORT IN THE GUT VERSUS LENGTH

Sodium transport into the serosal fluid versus length for 4 areas of the intestine in T. mossambica for fish A (o) and fish B (•).

Mandelstam (1960) measured tissue uptake, not transport, as did Stokes and Fromm (1964). Wilson and Wiseman (1954) used everted sacs but measured glucose by means of reducing value.

Mussachia and Neff (1963) found that in the ground squirrel all the areas of the upper intestine had an equal ability to absorb and transport glucose.

The effect of the inhibitor ouabain and the poison KCN of glucose and sodium transport in the different areas of the gut can be seen in Tables VIII and IX. In general, KCN and ouabain seem to have less effect on sodium than on glucose transport. Because of the problems involved with disposal of solutions which contain both radioisotopes and poisons, these experiments were not continued so that a statistical analysis could not be made. It is clear, however, that both mucosal ouabain and KCN inhibit glucose transport and that this inhibition is not complete, indicating that some glucose transport, and most of the sodium transport, occurs as a result of diffusion. The level of glucose which is transported across the pyloric area of the everted intestine of tilapia is 0.08 micro-moles/0.1 g wet weight for tissues inhibited by either KCN or ouabain. This is of the same order of magnitude of transport as the sugars such as mannitol, which also are presumed to enter the tissue through diffusion.

GLUCOSE AND SODIUM TRANSPORT: A study of the correlation

between ^{22}Na and glucose ^{14}C transport in everted gut sacs

of tilapia is presented in Figure 25. The results indicate

that for a change in glucose transport from 0.016 to 2.76

micro-moles/0.1 g wet weight, a sixteen-fold change. The

total range of sodium transport changes from 24.3 to 25.7

micro-moles/0.1 g wet weight, a change of only 1.4 micro-

moles/0.1 g wet weight. The change in sodium is not statis-

tically significant and may be due to random errors in

sampling. If all of the sodium transported had been re-

lated to glucose, one would expect a sixteen-fold increase

in sodium transport. If only a fraction of the sodium

transport was related to glucose transport, a minimum of

2.5 micro-moles/0.1 g wet weight change would be expected,

assuming a stochiometry of one sodium ion per glucose mole-

cule. The maximum range for all determinations in this ex-

periment was half of this value. The possibility of a 1:1

stochiometry for glucose and sodium transport could not be

eliminated, however, because of the high level of sodium

transported regardless of the presence or absence of glu-

cose.

This would seem to refute Asano (1964) who, in a study

of sodium and glucose transport using Ussings apparatus,

comes to the conclusion that glucose and sodium must both

be discharged across the serosal membrane by the carrier

molecule.

It seems clear that if active glucose transport shares
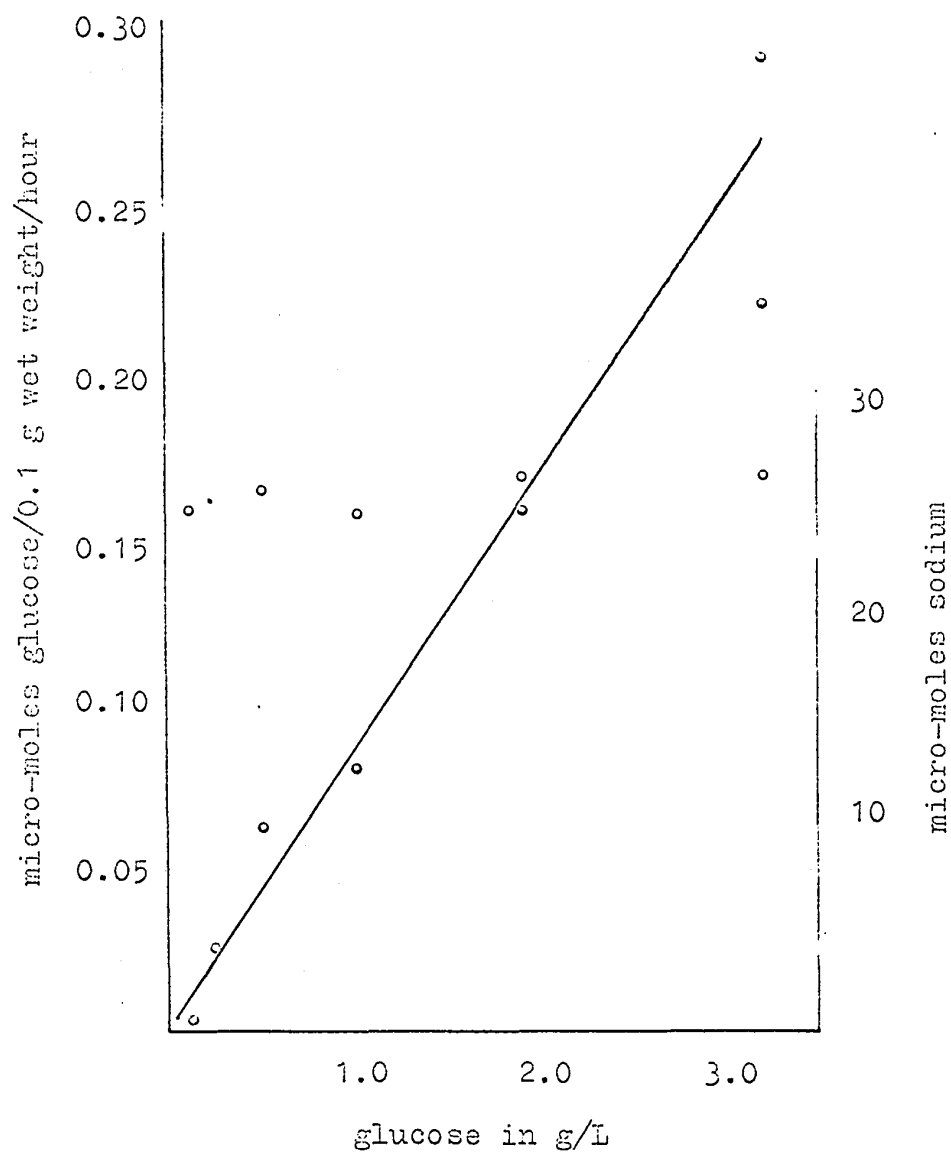
FIGURE 25. GLUCOSE AND SODIUM TRANSPORT VERSUS

MUCOSAL GLUCOSE CONCENTRATION

Transport of glucose [14]C (○) and [22]Na (○) into the everted gut sac of T. mossambica versus concentration of the mucosal fluid.

the same carrier molecule with sodium, this sodium does not enter the intracellular sodium pool and would not affect the sodium gradient between the cell and the medium.

If the sodium remained bound to the carrier for the return trip to the serosal surface it has already been pointed out that the sodium gradient could hardly be expected to provide the motive force for the carrier in both directions.

The hypothesis of a mobile sodium-carrier-glucose-molecule that moves from the mucosal to the serosal membrane surface and derives the energy for this movement from the sodium gradient between the cell and the medium is thus not consistent with the results of these experiments.

VI. ENZYMES AND TRANSPORT

SPECIFICITY AND SUGAR TRANSPORT: The specificity of the transport mechanism for specific sugars in other animals as reported by Crane (1960) and as found by Cordier (1955) for fish indicates that some step in the transfer mechanism must involve a highly selective "carrier" molecule, presumably a protein.

In Table X the specificity of sugar transport by everted gut sacs of tilapia shows that the mechanism for transport is highly selective: only glucose is actively transported against a gradient. Galactose is transported faster than can be accounted for by diffusion. The following sugars: mannose, fructose, glucuronic acid, L-fucose, zylose, gluconic acid, and mannitol do not enter the serosal fluid faster than can be accounted for by diffusion, using the rate of movement of glucose ^{14}C in poisoning experiments as the standard for diffusion.

These results are different from those found in other vertebrates as summarized by Crane (1960) where the order for the rate of absorption is galactose, glucose, mannose, fructose, and pentoses.

INHIBITION OF GLUCOSE TRANSPORT: The studies of inhibition of glucose transport by galactose are plotted in the form of a Lineweaver Burke graph in Figure 26 and it can be seen that a low concentration of galactose (5.55 meq/L acts as an accelerator of glucose transport. This is probably an

TABLE X

SPECIFICITY OF SUGAR TRANSPORT

A comparison between the transport of nine different sugars, in micro-moles/0.1 g wet weight/hour, in everted gut preparations of $\underline{T}$. $\underline{mossambica}$.

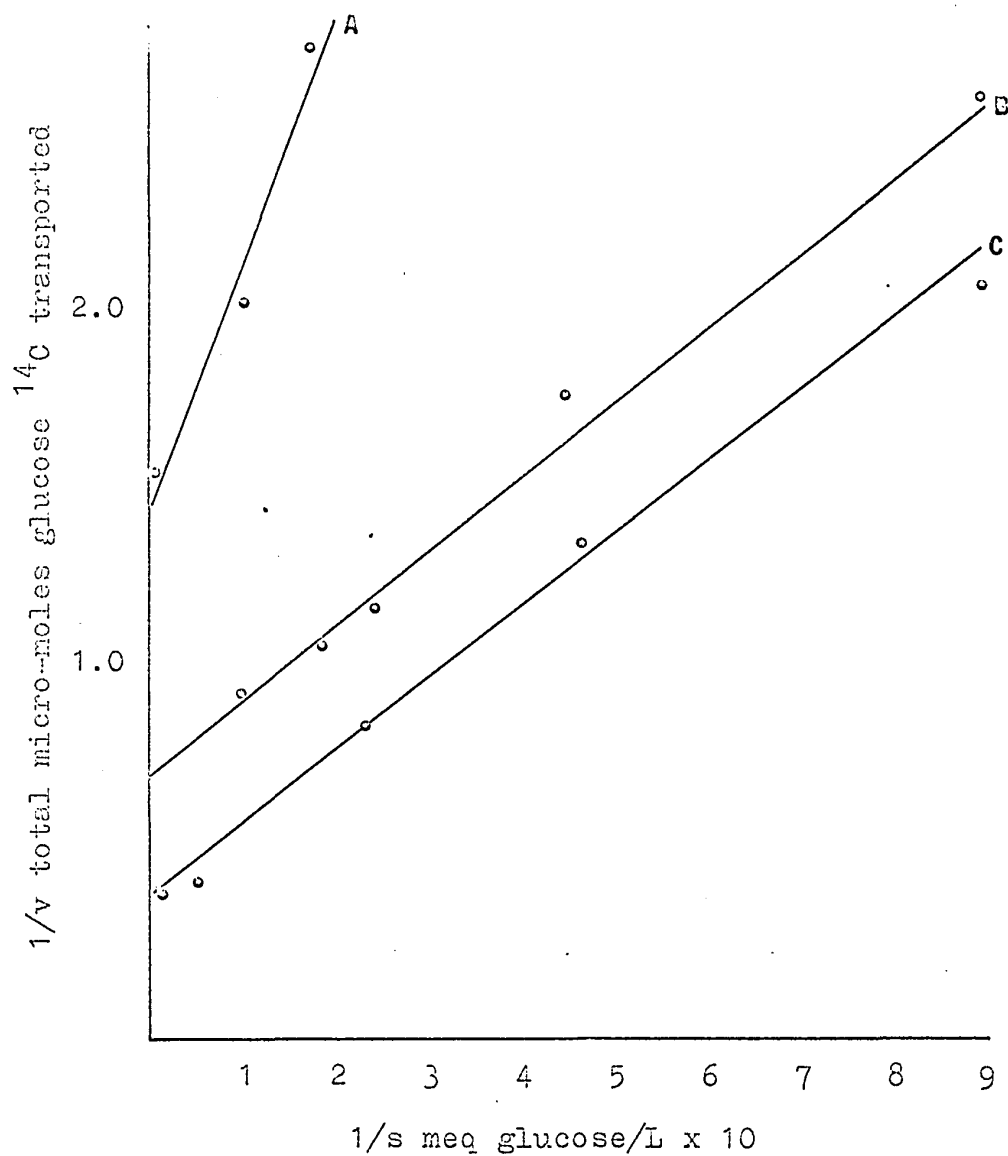|			
Glucose	0.2571	0.2472	0.2461
Galactose	0.1943	0.1113	0.1725
Glucose and poison	0.084	0.084	0.062
Zylose	0.0481	0.0467	0.0515
Glucose acid	0.0568	0.0311	0.0426
Glucuronic acid	0.0260	0.0440	0.0415
Mannose	0.0297	0.0284	0.0270
Mannitol	0.0177	0.0301	0.0275
L-fucose	0.0158	0.0193	0.0202
Fructose	0.0265	0.0152	0.0112

FIGURE 26.   INHIBITION OF GLUCOSE TRANSPORT BY GALACTOSE

Non-competetive inhibition of glucose ^{14}C transport into the serosal fluid by 55.5 meq/L galactose (A) in the mucosal fluid compared to normal glucose transport (B) and the acceleration of glucose transport by 5.55 meq/L galactose (C) in the mucosal fluid.

artifact due to variation in fish. The plot for glucose transport represents fish from one aquarium, while the experiments designed to show galactose inhibition represent the other two auqaria of fish, which seems to increase variability between experiments.

When high (5.55 meq/L) mucosal galactose concentrations are used, galactose acts as a pure non-competetive inhibitor of glucose transport, indicating that glucose and galactose do not share the same transport pathways.

This is in disagreement with the work of Crane and Mandelstam (1960) who conclude that in the hamster glucose and galactose shared the same transport mechanism. Other workers such as Fisher and Parsons (1954) have suggested that in the rat glucose and galactose have separate pathways.

A concentration of 55.5 meq/L galactose in the serosal fluid was not found to inhibit glucose transport into the serosal fluid of the everted intestinal sac. This indicates that inhibition of glucose transport by galactose occurs at the mucosal surface. These results are shown in Figure 27. GLUCOSE METABOLISM AND TRANSPORT: Serosal glucose concentration was shown to increase glucose transport into the serosal fluid in Figure 28. When the results of that experiment are compared with the effect of increasing concentration of glucose in the mucosal fluid it can be seen (Figure 28) that the ability to accelerate glucose transport
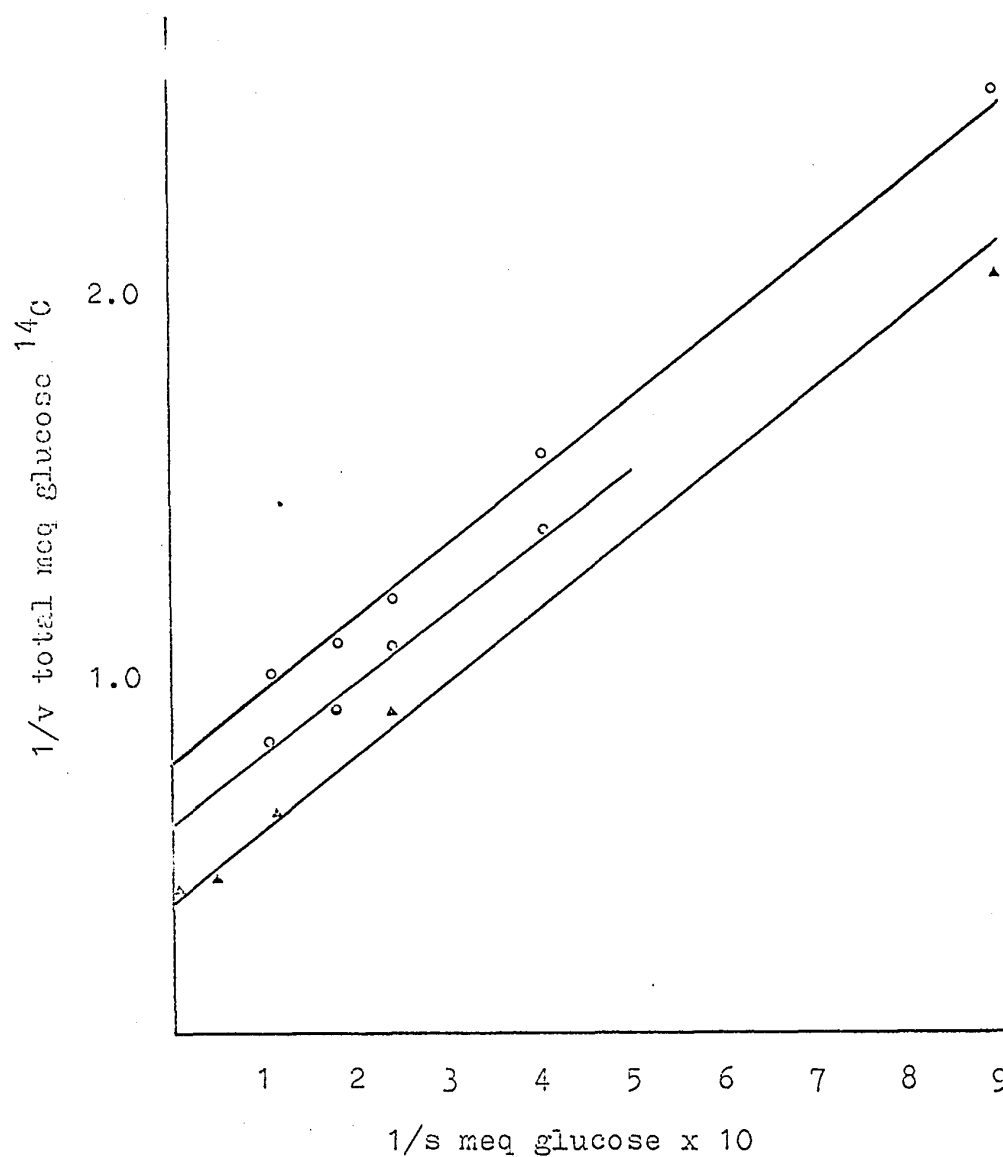
FIGURE 27. SEROSAL FLUID GALACTOSE AND GLUCOSE TRANSPORT

Apparent acceleration of glucose ^{14}C transport from the mucosal into the serosal fluid of T. mossambica by 5.55 meq/L galactose (▲) and 55.5 meq/L galactose (◦) in the serosal fluid compared to normal glucose transport (◦) with no serosal galactose.
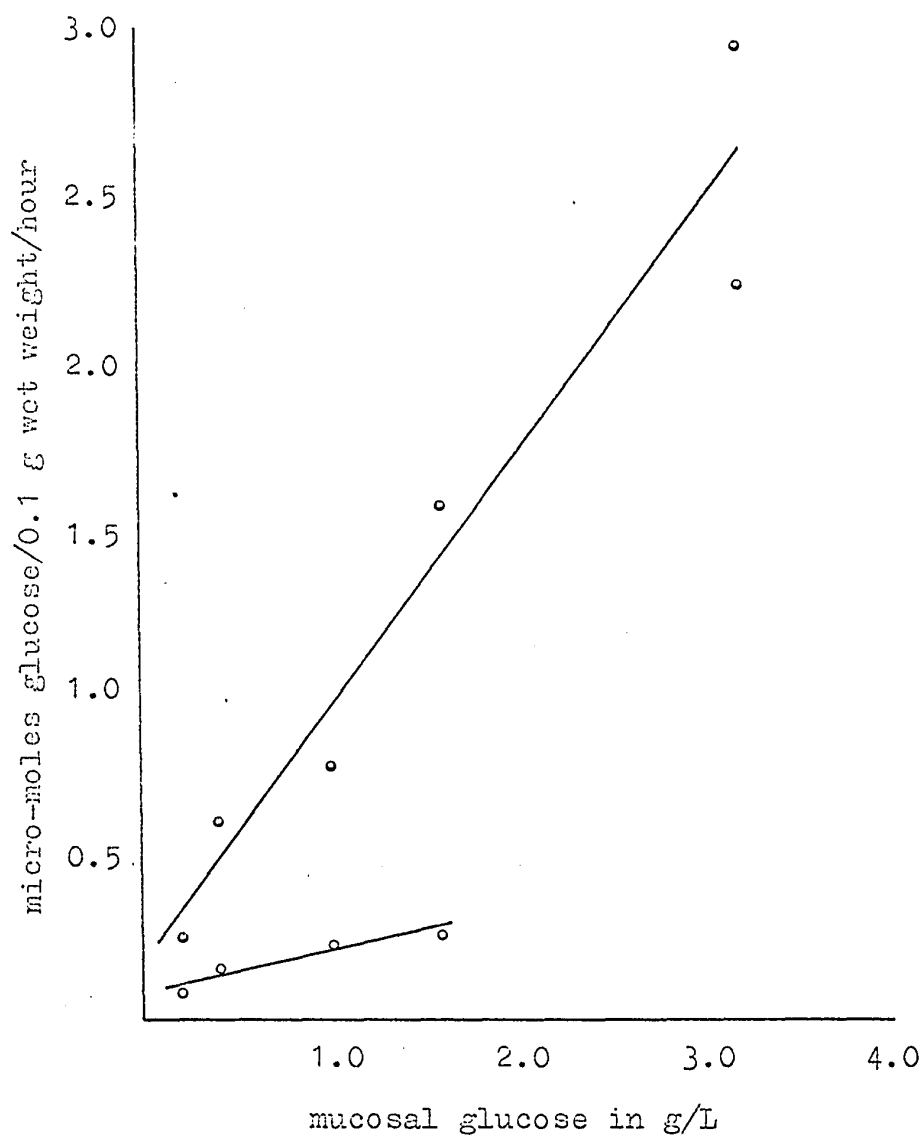
FIGURE 28. GLUCOSE TRANSPORT VERSUS MUCOSAL

OR SEROSAL GLUCOSE CONCENTRATION

Glucose ^{14}C transported into the serosal
fluid versus mucosal glucose concentration
(∘) and serosal glucose concentration (∘)
for T. mossambica.

is on a much lower order of magnitude for serosal glucose compared to mucosal glucose. From this it is inferred that the energy for glucose transport comes from glucose itself, but that the rate limiting step in glucose transport is on the mucosal luminal surface, and not inside the cell.

This is similar to the findings of Bingham (1966) who reports that serosal glucose accelerates methionium transport, indicating that the acceleration is due to metabolism of serosal glucose.

GLUCOSE TRANSPORT AND pH: The effect of pH on glucose transport is shown in Figure 29. There is a significantly greater glucose transport in the lower pH ranges of 5.6 than at the higher ranges. This does not indicate a pH optimum, but it does indicate that pH affects glucose transport. Csaky (1965) hypothesizes that the second step in glucose transport is the specific acceptance of glucose by sodium dependent membrane ATPase, which is the pump responsible for accumulation against a gradient; the results reported here are consistent with this hypothesis.
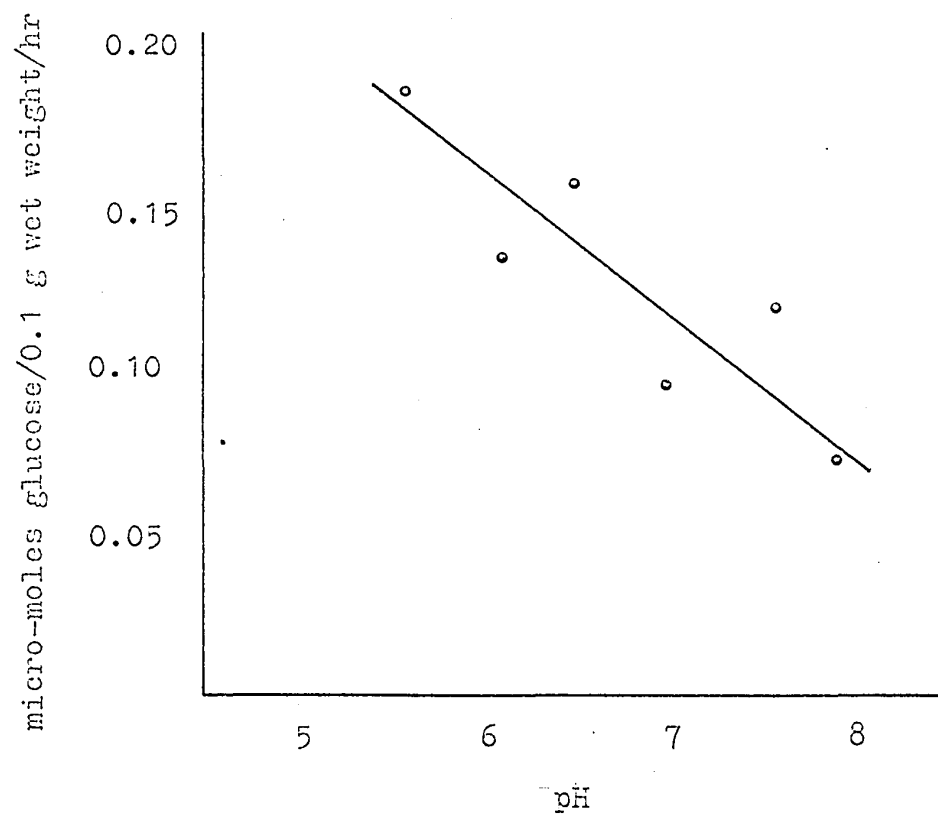
FIGURE 29. GLUCOSE TRANSPORT VERSUS pH

The effect of pH on glucose ^{14}C transport into the serosal fluid of T. moss-ambica (0.2 g/L mucosal glucose).

VII. A MODEL OF GLUCOSE TRANSPORT

PREVIOUS MODELS OF GLUCOSE TRANSPORT: Models of glucose
transport have been presented previously by Crane (1960,
1962, and 1965), Schultz and Zalusky (1964), Barry et al
(1965), and Benson and Rampone (1966).

The model of Barry et al (1965) is vague as to the
mechanism for glucose transport, but they offer evidence
that the transfer is electrogenic and causes ion movements.
They found no stochiometric relationship between glucose
and sodium transport.

In the other models of transport, summarized by the
model of Benson and Rampone (1966) and seen in Figure 30,
the movement of glucose across the membrane is linked with
sodium in an energy dependent step. Sodium, glucose, and a
carrier molecule are bound together and shuttle back and
forth across the membrane. Sodium is pumped out of the cell
by the sodium pump to create a sodium gradient which sup-
plies the energy for the carrier molecule movement to accu-
mulate glucose against a gradient. Disaccharides in the
brush border region are closely linked to the carrier mole-
cules so that after hydrolysis the products enter the cell
and not the mucosal luminal fluid.

Similar to the glucose transport theories of Crane et
al (1965) is the theory of glycine transport put forth by
Vidaver (1964). As in glucose transport, increased sodium
causes increased glycine transport. Glycine transport is

Glucose

Sucrose   Na⁺

Pr+Gl

Gl + Na⁺   Digestive
                      area

mobile
carriers   Diffusion
                      barrier

Gl + Na⁺   ATP
              ADP

Glucose
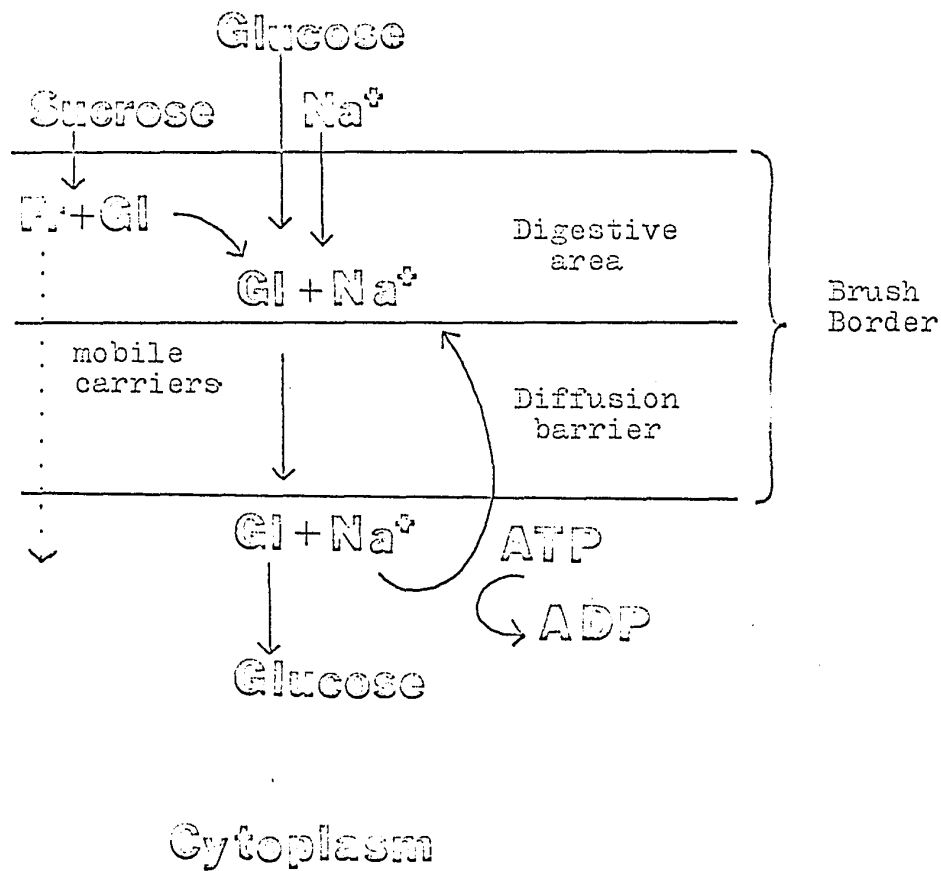
Cytoplasm

Brush
Border

FIGURE 30.   MODEL OF GLUCOSE TRANSPORT IN PHYSIOLOGICAL
        REVIEWS BY BENSON AND RAMPONE (1966)

energy independent and depends upon a sodium electro-chemical gradient. Counter transport of glycine can be demonstrated when the sodium gradient is reversed, either by reversing the sodium concentrations or by a Donnan effect. A stochiometry of 2:1 between sodium and glycine was found.

It is not reasonable to expect to criticize the work of Vidaver (1964) based upon results obtained using a completely different experimental design. However, in the present study energy from glucose metabolism was shown to be involved in glucose transport by the addition of glucose to the serosal fluid and by the effect of poisons on glucose transport. Counter transport and transport of glucose occurred at the same time, and counter transport was demonstrated before a reversal of sodium gradient occurred in experiments with tilapia. These results indicate that glucose and sodium do not enter the cell by means of a downhill electrochemical gradient and are experiments that Vidaver did not perform.

ANOTHER MODEL OF GLUCOSE TRANSPORT: Because the results of the experiments with tilapia are different in some respects from the results of other workers, a new model for glucose transport is presented in Figure 31.

The first step in glucose transport is the binding of the molecule to an enzyme at the mucosal membrane surface. This is better documented for disaccharides than monosacoharides, thanks to the works of Chain et al (1960), Crane
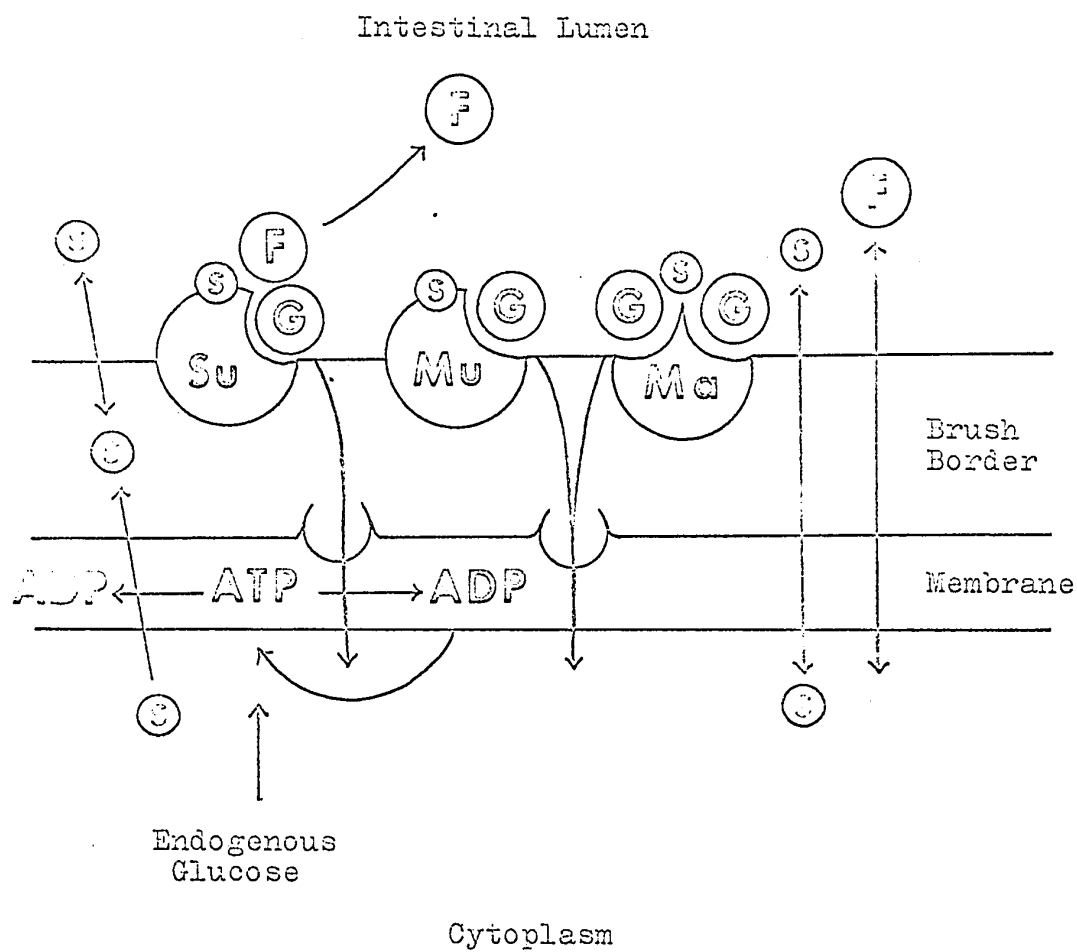
FIGURE 31. A HYPOTHETICAL MODEL OF GLUCOSE TRANSPORT

S = sodium, Su = sucrase, Ma = maltase, Mu = mutaro-
tase, F = fructose, G = glucose.

and Miller (1961), Dahlqvist and Borgstrom (1961), Semenza
et al (1964), and Dahlqvist and Nordstrom (1966) who have
found that disaccharides enter the cell faster than can be
accounted for by the rate of hydrolysis detectable in the
lumen. Glucose oxidase, an enzyme that would attack free
glucose, was unable to prevent intracellular accumulation
of glucose when maltose or glucose-1-PO_4 was in the luminal
medium. Crane and Miller (1961) state that this indicates
that the hydrolysis of the disaccharide occurred within the
cell. A more logical explanation, in view of the fact that
the PO_4 is known to remain in the mucosal fluid, is that the
substrate is bound to the enzyme at the mucosal surface,
hydrolyzed, and accepted for transport across the membrane
without re-entering the luminal field. As long as the pro-
ducts of hydrolysis remain bound they are immune to attack
by the glucose oxidase.

The work of Keston (1963) and Bailey and Pentchev
(1964) indicates that the enzyme mutarotase may be similar-
ly involved as a first step in glucose transport.

Sodium is necessary for the ansorption of glucose be-
cause it is a co-factor for these enzymes and becomes bound
to the enzyme with the glucose. This binding, similar to
the binding of glucose and sodium to Crane's carrier mole-
cule, may be responsible for the change in membrane poten-
tial during transport of glucose.

The decreased amount of glucose transport seen in the

experiments with tilapia when the sodium level of the medium was below 100 meq/L is probably due to the sodium co-factor requirement for enzymatic activity. It may also be due to a loss of intracellular sodium when the extracellular sodium falls below 100 meq/L. This loss would theoretically be more critical because of the small amount of sodium within the cell, and would effect intracellular enzymes, such as sodium dependent ATPase which needed sodium as a co-factor. The point at which the intracellular sodium concentration becomes sensitive to external sodium concentration should be investigated.

Glucose and sodium do not share a common carrier into the cell in this model because glucose transport has been shown to be energy dependent, counter transport and transport occur at the same time, and counter transport begins before there is a reversal of the sodium gradient. This is, however, not clear-cut, as the study of the effect of glucose concentration on sodium and glucose transport showed. While most of the sodium transport is clearly not related to glucose transport, it is possible that there is a one to one stochiometry between glucose and sodium transport.

The mechanism suggested for glucose transport in this model is a form of molecular phagocytosis similar to that demonstrated by Palay and Karlin (1959) for fats and by Chapman-Anderson and Holter (1964) for proteins. The probability of phagocytosis and hence transport is dramatically

increased for those sugars bound to enzymes on or at the
membrane surface. These enzymes, which are substrate speci-
fic and in many cases require sodium for activity are simi-
lar to the "carrier molecules" in Crane's model except that
they do not actually move through the membrane. The phago-
cytosis of glucose, perhaps including some sodium ions, by
the membrane adjacent to the enzyme sites may be thought of
as a "pore" that opens in response to stimulation of the
membrane following attachment to an enzyme without actually
endangering membrane integrity.

Those sugars not bound to surface enzymes would have
a much lower probability of being phagocytized because they
would not normally be in a position to stimulate the mem-
brane. Glucose can build up against a concentration gra-
dient because there are no enzymes on the intracellular
surface to increase the probability of transport.

The increased membrane activity associated with the
molecular phagocytosis of glucose would help to explain the
change in potential during transport found by Schultz and
Zalusky (1964). This increased activity would also help
account for the energy requirements for glucose transport
as seen in the experiments using poisons.

Endogenous glucose presumably increases glucose trans-
port through increased metabolism and energy available for
phagocytosis or restitution of the membrane.

Because KCN did not inhibit the rate of sodium

accumulation in the serosal fluid the model indicates that sodium enters the cells primarily through diffusion. The similarity in the rate of transport of sodium throughout the inyestine also indicates that sodium movement is related to surface area and diffusion rather than enzyme level.

The different rates of transport of glucose in the intestine would presumably reflect different amounts of enzymes able to bind glucose to make it available for phagocytosis. Indeed, the distribution of mutarotase and maltase parallel the areas of active absorption of glucose, according to Keston (1961) and Dahlqvist and Nordstrom (1966).

The rate limiting factor of transport is not only specific, as shown by the study of the rates of transport of eight other sugars besides glucose, but it is on the mucosal membrane surface, as indicated by the great increase in transport caused by mucosal glucose compared to serosal glucose. The fact that mucosal galactose inhibited glucose transport while serosal galactose did not also indicates that the rate limiting factor is on the mucosal surface. The specificity and rate limiting properties on the mucosal surface are probably the enzymes which partially bind glucose to make it available for phagocytosis.

The failure of changes in diet or environment to affect glucose or galactose transport indicates that the rate

limiting factor of glucose transport is not an inducible enzyme. Sodium, which probably enters by diffusion, would therefore not be expected to be affected by changes in diet or environment.

This model of glucose transport is admittedly speculative, but it is not radically new in that no mechanisms unknown in other cells are presented. Rather, it is an attempt to look at the problem of glucose transport from a new approach. The most recent paper of Crane and Lyon (1966) states, "Full computer analysis (of the results) will require some months further time and final results will be reported in a subsequent publication."

When common people are faced with problems they cannot solve, they turn to God. Scientists turn to mathematics, perhaps knowing that if they cannot solve the problem, they can at least present it in such a way that the results are known only to themselves and their God.

BIBLIOGRAPHY

Alvarado, F. 1965. The relationship between Na$^+$ and the active transport of arbutin in the small intestine. Biochim. Biophys. Acta. 109: 478-494.

Alvarado, F. 1966. Transport of sugars and amino acids in the intestine: Evidence for a common carrier. Science. 151: 1010-1012.

Alvarado, F. 1966. D-Zylose active transport in the hamster small intestine. Biochim. Biophys. Acta. 112: 292-306.

Alvarado, F. and R.K. Crane. 1964. Phenylglycoside transport and its possible relationship to phlorizin inhibition of the active transport of sugars by the small intestine. Biochim. Biophys. Acta. 93: 116-135.

Asano, T. 1964. Metabolic disturbances and short-circuit current across intestinal wall of rats. Am. J. Physiol. 207: 415-422.

Bailey, J.M. and P.G. Pentchev. 1964. Inhibition of kidney and intestinal mutarotase by actively absorbed sugars. Bioch. and Biophys. Res. Comm. 14: 161-164.

Bailey, J.M. P.G. Pentchev and J. Woo. 1965. Distribution of a mutarotase activity in rat tissues and possible function in active transport of sugars. Biochim. Biophys. Acta. 94: 124-129.

Barry, R.J.C., D.H. Smyth and E.M. Wright. 1965. Short circuit current and solute transfer by rat jejunum. J. Physiol. London. 181: 410-431.

Bassler, K.H., G. Stein und W. Belzer. 1966. Zylitstoffwechsel und zylitresorption. Biochem. Zeitsh. 346: 171-185.

Bell, D.J. 1962. Natural monosaccharides and oligosaccharides in Comparative Biochemistry. ed. by M. Florkin and H.S. Mason. 3: 288-342.

Benson, J.A. and A.J. Rampone. 1966. Gastrointestinal absorption. Physiol. Rev. 28: 201-226.

Berger, L.R. and D.M. Reynolds. 1958. The chitinase system of a strain of Streptomyces griseus. Biochim. Biophys. Acta. 29: 522-534.

Bihler, I. and R.K. Crane. 1962. Influence of several cations and anions on the transport of sugars in vitro by various preparations of hamster small intestine. Biochim. Biophys. Acta. 59: 78-93.

Bingham, J.K., H. Newey and D.H. Smyth. 1966. Interaction of sugars and amino acids in intestinal transfer. Biochim. Biophys. Acta. 130: 281-284.

Bosackova, J. and R.K. Crane. 1965. Cation inhibition of active sugar transport and ^{22}Na influx into hamster small intestine in vitro. Biochim. Biophys. Acta. 102: 423-436.

Bosackova, J. and R.K. Crane. 1965. Intracellular sodium concentration and active sugar transport by hamster small intestine in vitro. Biochim. Biophys. Acta. 102: 436-441.

Brown, K.M. and F. Moog. 1967. Invertase activity in the intestine of the developing chick. Biochim. Biophys. Acta. 132: 185-187.

Carlisky, N.J. and K.C. Huang. 1962. Glucose transport by intestinal tissue of the dogfish. Proc. Soc. Exp. Biol. Med. 109: 405-408.

Chapman-Anderson, C. and H. Holter. 1964. in Transport through biological membranes. T.Z. Csaky. 1965. Ann. Rev. Physiol. 27: 417.

Chain, E.B., K.R.L. Mansford and E. Pocchiari. 1960. The absorption of sucrose, maltose, and higher oligosaccharides from the isolated rat intestine. J. Physiol. 154: 39-51.

Clausen, T. 1965. The relationship between the transport of glucose and cations across cell membranes in isolated tissues. Biochim. Biophys. Acta. 109: 164-171.

Cordier, D. and J. Chanel. 1953. Influence de la tension d'oxygene sur l'absorption intestinale des solutions de pentoses et d'hexoses chez la Rascasse (Scorpaena porca). J. Physiol. Paris. 45: 91-93.

Cordier, D. and J.F. Worbe. 1954. Etudes sur l'absorption intestinale des hexoses et des pentoses chez la grenouille (Rana esculenta). J. Physiol. Path. Gen. 46: 318-322.

Cordier, D. and A. Maurice. 1955. Absorption intestinale des
    pentoses chez la tanche (Tinca vulgaris). Compt. Rend.
    Soc. Biol. Paris. 149: 732-734.

Cordier, D. and J.F. Worbe. 1955. Etude sur l'absorption in-
    testinale des hexoses et des pentoses chez la grenouille
    (Rana esculenta). Compt. Rend. Soc. Biol. Paris. 149:
    110-112.

Cordier, D. and J.F. Worbe. 1956. Modification de l'absorp-
    tion intestinale du glucose chez la grenouille (Rana
    esculenta) sous l'influence de melanges gazeux pauvres
    en oxygene et enrichis en anhydride carbonique. Compt.
    Rend. Soc. Biol. Paris. 150: 1960-1963.

Cordier, D. and J.F. Worbe. 1956. Recherches sur la vitesse
    de l'absorption intestinale du glucose chez la gren-
    ouille (Rana esculenta) en fonction de la tension de
    l'oxygene dans le miliew exterieur. Compt. Rend. Soc.
    Biol. Paris. 150: 1204-1205.

Cordier, D., A. Maurice and J.F. Worbe. 1957. Influence de
    la concentration des solutions de sucrea sur la vitesse
    de l'absorption intestinale chez les poecilotheremes.
    J. Physiol. Path. Gen. 49: 104-107.

Crane, R.K. 1960. Intestinal absorption of sugars. Physiol.
    Rev. 40: 789-825.

Crane, R.K. 1962. Intestinal transport of sugars. Fed. Proc.
    21: 891-895.

Crane, R.K. 1965. Na$^+$ dependent transport in the intestine
    and other animal tissues. Fed. Proc. 24: 1000-1006.

Crane, R.K., G. Forstner and A. Eichholz. 1965. Studies on
    the mechanism of the intestinal absorption of sugars.
    Biochim. Biophys. Acta. 109: 467-477.

Crane, R.K. and I. Lyon. 1966. Studies on transmural poten-
    tials in vitro in relation to intestinal absorption.
    Biochim. Biophys. Acta. 112: 278-291.

Crane, R.K. and P. Mandelstam. 1960. The active transport of
    sugars by various preparations of hamster intestine.
    Biochim. Biophys. Acta. 45: 460-476.

Csaky, T.Z. and M. Thale. 1960. Effect of ionic environment
    on the intestinal sugar transport. J. Physiol. 151:
    59-65.

Csaky, T.Z. 1965. Transport through biological membranes. Ann. Rev. Physiol. 27: 415-450.

Csaky, T. Z. and G. W. Fernald. 1960. Absorption of 3-methyl-glucose from the intestine of the frog Rana pipiens. Am. J. Physiol. 198: 445-448.

Curran, P.F. 1965. Ion transport in intestine and its coupling to other transport processes. Fed. Proc. 24: 993-999.

Dahlqvist, A. and B. Borgstrom. 1961. Digestion and absorption of disaccharides in man. Biochem J. 81: 411-418.

Dahlqvist, A. and C. Nordstrom. 1966. The distribution of disaccharidase activity in the villi and crypts of the small intestinal mucosa. Biochim. Biophys. Acta. 113: 624-626.

Dawson, A.G., H. Newey and D.H. Smyth. 1965. Effect of glucose on different amino acid transfer systems in rat intestine. J. Physiol. London. 179: 56-57.

Diedrich, D.F. and L. Anderson. 1960. Galactose 1-PO$_4$ in the intestinal tissue of the rat during galactose absorption. Biochim. Biophys. Acta. 45: 490-498.

Duerdoth, J.K., H. Newey, P.A. Sanford and D.H. Smyth. 1965. Stimulation of intestinal fluid transfer by mannose and fructose. J. Physiol. London. 176: 23-24.

Faitelberg, R.O. 1961. Present status on the question of carbohydrate absorption by the intestines. Fiziol. Zhur. Akad. Nauk Ukrain R.S.R. 5: 246-260.

Fisher, R.B. and D.S. Parsons. 1953. Galactose absorption from the surviving small intestine of the rat. J. Physiol. 119: 224-232.

Fox, A.M. 1961. Active transport of ^{14}C D-glucose by turtle small intestine. Am. J. Physiol. 201: 295-297.

Fox, A.M. 1961. Transport of monosaccharides by intestinal segments of the painted turtle Chrysemys picta. Comp. Bioch. Physiol. 3: 285-296.

Fox, A.M. 1962. Active transport of D-glucose and 3-O-methyl glucose by turtle intestine. J. Iowa acad. Sci. 69: 600-605.

Fox, A.M. 1965. Absorption of fatty acids by isolated segments of turtle small intestine. Comp. Bioch. Physiol. 14: 553-556.

Fox, A.M. and X.J. Mussachia. 1960. In vitro studies of intestinal absorption in the turtle Chrysemys picta. Fed. Proc. 19: 82.

Fox, A.M., S. Thiers, L. Rosenberg and S. Segal. 1964. Ionic requirements for amino acid transport in the rat kidney cortex slice. Biochim. Biophys. Acta. 79: 167-176.

Hiatt, R., S. Boroughs and S. Townsley. 1956. Radioisotope uptake in marine organisms. Annual report 1955-1956 AT(04-3)-56.

Hollands, B.C.S. and M.W. Smith. 1964. Phosphatases of the goldfish intestine. J. Physiol. 175: 131-137.

House, C.R. and K. Green. 1963. Sodium and water transport across isolated intestine in a marine teleost. Nature. 199: 1293-1294.

Huang, K.C. and W.R. Rout. 1967. Intestinal transport of sugars and aromatic amino acids in the killifish, Fundulus heteroclitus. Am. J. Physiol. 212: 799-803.

Jorgensen, C.R., B.R. Landau and T.H. Wilson. 1961. A common pathway for sugar transport in hamster intestine. Am. J. Physiol. 200: 111-116.

Kabat, E. A. and M.M. Meyer. 1966. Experimental Immunochemistry. Charles Thomas. 2nd. ed: 525.

Keston, A.S. 1963. Mutarotase and sugar transport by kidney and intestine. Fed. Proc. 22: 416.

Kipnis, D.M. and J.E. Parrish. 1965. Role of Na^+ and K^+ on sugar and amino acid transport in striated muscle. Fed. Proc. 24: 1051-1059.

Lawrence, A.L. 1963. Specificity in sugar transport by the small intestine of the bullfrog Rana catesbeiana. Comp. Bioch. Physiol. 9: 69-73.

Long, C. 1965. Biochemists Handbook. D. van Nostrand Company: 28.

Lyon, I. and R.K. Crane. 1966. Studies on transmural potentials in vitro in relation to intestinal absorption. II an effect of ouabain on glucose-dependent increment of transmural potential of rat small intestine. Biochim. Biophys. Acta. 126: 146-153.

Lyon, I. and R.K. Crane. 1967. Studies on in vitro transmural potentials in relation to intestinal absorption K⁺ inhibition of sodium-dependent transmural potential of rat small intestine. Biochim. Biophys. Acta. 135: 61-73.

Miller, D. and R.K. Crane. 1961. The digestive function of the epithilium of the small intestine. Biochim. Biophys. Acta. 52: 281-298.

Mussachia, X.J. 1960. Some characteristics of active transport of sugars by intestinal segments of Ameiurus nebulosus. Biol. Bull. Woods Hole. 119: 328-329.

Mussachia, X.J. 1961. Absorption and active transport of sugar (D-glucose) in segments of fish intestine. Fed. Proc. 20: 244.

Mussachia, X.J. and S.S. Fisher. 1960. Comparative aspects of active transport of D-glucose by in vitro preparations of fish intestine. Biol. Bull. Woods Hole. 119: 327-328.

Mussachia, X.J. and D.D. Westhoff. 1962. Nitrogen inhibition of active absorption of D-glucose in fish intestine. Biol. Bull. Woods Hole. 123: 507.

Mussachia, X.J. and D.D. Westhoff. 1962. Phlorizin inhibition of active absorption of D-glucose in fish intestine. Biol. Bull. Woods Hole. 123: 507.

Mussachia, X.J. and D.D. Westhoff. 1963. Comparisons of active absorption in vitro in fish intestine. Fed. Proc. 22: 227.

Mussachia, X.J. and R.A. Hines. 1963. Intestinal absorption of D-glucose in various species of fish. Biol. Bull. Woods Hole. 125: 386.

Mussachia, X.J. and S.S. Neff. 1963. Active absorption of D-glucose by intestinal segments of the ground squirrel Citellus tridecemlineatus. Comp. Bioch. Physiol. 9: 37-40.

Mussachia, X.J., D.D. Westhoff and S.S. Fisher. 1961. A comparative study of D-glucose uptake and transport by intestinal segments of Stenotomus versicolor and Ameiurus nebulosus. Biol. Bull. Woods Hole. 121: 400-401.

Mussachia, X.J., D.D. Westhoff and S.S. Neff. 1964. Active transport of D-glucose by intestinal segments in vitro of Ictalurus nebulosus. Biol. Bull. Woods Hole. 126: 291-301.

Mussachia, X.J., D.D. Westhoff and R. Van Haaren. 1966. Active transport of D-glucose in intestinal segments in vitro of the scup, Stenotomus versicolor and the puffer, Spheroides maculatus. Comp. Biochem. and Physiol. 17: 93-106.

Newey, H. and D.H. Smyth. 1964. Effects of sugars on intestinal transfer of amino acids. Nature. 202: 400-401.

Palay, S.L. and L.J. Karlin. 1959. in The Textbook of Biochemistry. E.S. West, W.R. Todd, H.S. Mason and J.T. Van Bruggen. MacMillan Co. 4th ed: 529.

Pope, J.L., T.M. Parkinson and J.A. Olson. 1966. Action of bile salts on the metabolism and transport of water soluble nutrients by perfused rat jejunum in vitro. Biochim. Biophys. Acta. 130: 218-232.

Roe, J.H., J.M. Bailey, R.R. Gray and J.N. Robinson. 1958. Complete removal of glycogen from tissue by extraction with cold trichloracetic acid solution. J. Biol. Chem. 233: 1244-1246.

Rosenweig, N.S., A.E. Cocco and T.R. Hendrix. 1965. A comparison of the effect of sodium and lithium on the absorption of glucose and zylose in vivo. Biochim. Biophys. Acta. 109: 312-313.

Rout, W., D.S.T. Lin and K.C. Huang. 1965. Intestinal transport of glucose in flounder fish. Proc. Soc. Exp. Biol. Med. 118: 933-938.

Saunders, S.J. and K.J. Isselbacher. 1965. Inhibition of intestinal amino acid transport by hexose. Biochim. Biophys. Acta. 102: 397-409.

Schatzmann, H.j. 1965. The role of Na^+ and K^+ in the ouabain inhibition of Na^+ and K^+ activated membrane Adenosine tripphosphatase. Biochim. Biophys. Acta. 94: 89-101.

Schoffeniels, E. 1964. Cellular aspects of active transport in Comparative Biochemistry. M. Florkin. Acd. Press. 7137.

Schultz, S.G. and R. Zalusky. 1964. The interaction between active sodium and active sugar transport. J. Gen. Physiol. 47: 1043-1059.

Schultz, S.G. and R. Zalusky. 1965. Interactions between active sodium transport and active amino acid transport in isolated rabbit ileum. Nature. 205: 292-294.

Semenza, G., R. Tosi, M.C. Vallotton-Delachaux and E. Mulhaupt. 1964. Sodium activation of human intestinal sucrose and its possible significance in the enzymatic organization of brush borders. Biochim. Biophys. Acta. 89: 109-116.

Smith, M.W. 1964. The in vitro absorption of water and solutes from the intestine of the goldfish Carassius auratus. J. Physiol. 175: 38-49.

Snedecor, G.W. 1956. Statistical Methods. 5th ed. Iowa State Univ. Press.

Stokes, R.M. and P.O. Fromm. 1964. Glucose absorption and metabolism by the gut of rainbow trout. Comp. Bioch. Physiol. 13: 53-69.

Stokes, R.M. and P.O. Fromm. 1965. Effect of chromate on glucose transport by the gut of rainbow trout. Physiol. Zool. 38: 202.

Vidaver, G.A. 1964. Glycine transport by hemolyzed and restored pigeon red cells. Biochem. 3: 795-799.

Vidaver, G.A. 1964. Mucate inhibition of glycine entry into pigeon red cells. Biochem. 3: 799-803.

Vidaver, G.A. 1964. Some tests of the hypothesis that the sodium gradient furnishes the energy for glycine active transport by pigeon red cells. Biochem. 3: 803-808.

Westenbrink, H.G.K. 1936. Uber die spezifitat der resorption einer monosen aus dem darm der ratte und der taube. Arch. Neerl. Physiol. 21: 433.

Wilson, T.H. 1957. In vitro studies on intestinal absorption of fish. Biol. Bull. Woods. Hole. 113-362.

Wilson, T.H. 1962. <u>Intestinal</u> <u>Absorption</u>. Saunders.

Wilson and Wiseman. 1954. The use of everted sacs of small intestine for the study of transference of substances from the mucosal to the serosal surface. J. Physiol. 123: 116-125.